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Biochemical genetic variation in the freshwater Copepoda: Evolutionary consequences of passive dispersal in freshwater zooplankton.

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BIOCHEMICAL GENETIC VARIATION IN THE FRESHWATER COPEPODA: EVOLUTIONARY CONSEQUENCES OF PASSIVE DISPERSAL IN FRESHWATER ZOOPLANKTON

by

Marc Gerald Boileau

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- please omit Appendix II, pp. 168-179.

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ABSTRACT

Although the copepod crustaceans have been studied for more than 200 years, the freshwater forms have always been less intensely studied than their marine counterparts and little is known of their genetics. This is especially true in North America, where few studies have aimed to advance knowledge of the systematics and evolution of this group.

This study investigated 24 North American species within 2 orders in the Copepoda, representing a substantial proportion of the known fauna. Biochemical variation was best explained by simple Mendelian segregation of alternate alleles and from 12.5% to 53.2% of loci were polymorphic with 1.12-2.0 alleles per locus on average. The genetic variation was unaffected by sex linkage, autosomal linkage disequilibrium or four important physical parameters (absorbance @ 350nm, alkalinity, conductivity and pH).

Detailed analyses of copepod populations at arctic locations revealed that gene frequencies within populations were stable between years but differed substantially among populations only a few meters apart. However, the extent of gene frequency differentiation was similar in species with restricted local distribution to those which were widespread. In fact, gene frequency divergence among populations was common in passively dispersed pond dwelling invertebrates, and not correlated to dispersal capacity criteria as expected from equilibrium population genetics

theory.

On a broader scale, the patterns of gene frequency variation in two species distributed widely in the arctic were markedly different. Heterocope septentrionalis was most variable in western arctic locations, near Beringia, and significantly more so than in eastern locations. This difference was due to the absence, in eastern populations, of many alleles from polymorphic loci. On the other hand, fixed allelic differences were common among microallopatric populations of Hesperodiaptomus arcticus sensu lato. Indeed, such differences were observed within single ponds in some cases and were best explained by the co-occurrence of reproductively isolated species. Three genetically distinct taxa are present within H. arcticus sensu lato and two new species are proposed, Hesperodiaptomus nearcticus and Hesperodiaptomus churchillensis. A detailed analysis of these two taxa, where they co-occur, confirmed ecological and life-history differences which promote their isolation.

DEDICATION

To my dear wife Liz who endured patiently and never ceased to be dedicated to my projects. To Deric and Michael, because I was away so much of the time while I completed my studies. Their loyalty was instrumental to the success of this research and I am forever in their debt.

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Arctic specimens were collected by permission from the Government of the Northwest Territories (permit numbers 5127 [1985], 6053 [1986], 7079 [1987], 8023 [1988]), Yukon Territorial Parks (105-1-88 [1988]) and Parks Canada (B88-06, J88-14, NY88-10). Field collections from Saskatoon were made by Guy Melville who also "worried" them through the air freight system on my behalf. Magi Beaton, John Havel, Paul Hebert and Steve Schwartz also seemed to provide an endless

supply of animals for me. Paul and Steve also collected most of the Igloolik data contained in Chapter 4 and Brenda Hann provided her Simocephalus data.

Neil Billington generously provided his house in England and Bob Ward his lab at Loughborough University during 1986. I was hosted by Ulrich Einsle at the Institut fur Seenforschung und Fischereiwesen, Konstanz Germany and Geoffry Fryer at the Freshwater Biological Association, Ambleside England in 1986 and both were generous with their time.

Jim and Janet Hardy gave me northern hospitality for 4 days in Paulatuk in 1986. Frank Mallory (Laurentien University) provided a much needed trike and hospitality in Eskimo Point during 1988. I was assisted by David Craig in Rankin Inlet in 1988 and Chris Wilson willingly bailed me out (at 2 a.m.) while we gelled the animals that didn't survive the flight from Rankin.

Stewart Anderson, Richard Smith and Geoffry Fryer provided information on the collecting localities. I enjoyed the company of Magi Beaton at Tuktoyaktuk in 1988 and Neil Billington (too many years to remember) in Churchill. Peter Gajda conducted an excellent field study of Hesperodiaptomus for me in 1988.

Finally, I shared my triumphs and set-backs with my lab colleagues especially Dave Barker, Magi Beaton, Neil Billington, Roy Danzmann, Terrie Finston, John Havel, David Innes, Mary Murdoch, Steve Schwartz, Dave Stanton and Larry Weider who were always willing to contribute ideas and friendship.

PREFACE

Their ancestors had gotten isolated on an island
(it's explained by scientists after observation so close
that in their dreams they consulted with 'em)
And started getting smaller to fit that island
Like the reasoning power of a Ph.D.
When he's specialized and specialized down to the smallest
specialization of his specialization
Til only one braincell is active, and that sleepily...
Perhaps (one theory happens to go)
The hunting got richer and richer as they got smaller
and smaller
Til now they spend happy weeks in a rabbit's ear
Hunting fleas like cavemen after mammoths.

"The Microscopic Army Ants of Corsica" Milton Acorn 1972

The uniqueness of organisms, which become isolated, has attracted the attention of poets and scientists alike. Evolutionary biologists have been fascinated by island inhabitants because in them it is hoped that the subtle steps of species divergence might be observed. Recently, much of this interest has focussed on the genetic differences associated with reduced gene flow and complete isolation of populations.

Although the connection between island isolation and aquatic organisms has not always been appreciated, this group may be the best with which to study the genetic mechanisms which accompany population isolation. In particular, the passively dispersed pond dwelling copepods possess the ample genetic variation required for such studies. For example, studies on natural populations of actively dispersing terrestrial organisms, whose macrogeographic dispersal was known, could not detect

significant losses in genetic variation at multiple allozyme loci. Yet the repeated founder events due to passive dispersal of pond copepods revealed such significant losses in the present study. It is hoped that future studies on aquatic organisms can extend our knowledge of the evolutionary consequences of reduced gene exchange.

Genetic studies can also serve to improve the systematic understanding of the Copepoda which has remained neglected for thirty years in North America. Indeed, many of the much needed evolutionary studies may have to await a better systematic understanding of the copepods because the correct delimitation of species is fundamental to the examination of gene flow among populations.

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Chapter I

General Introduction

Variation in the extent of gene flow among spatially separated populations has long been recognized for its potential to influence the evolution of organisms (Mayr, 1963; Carlquist, 1966). In theory, restricted gene flow among populations alone will lead to genetic differentiation of selectively neutral genes (Wright, 1931, 1940, 1943, 1951) and genetic differentiation, if sustained for long enough, can lead to reproductive isolation (Mayr, 1963). However, there has been no general agreement on the importance of this phenomenon in natural populations (Ehrlich and Raven, 1969; Jackson and Pounds, 1979), in part due to the difficulty in estimating gene flow. There remains a need for evolutionary biologists to estimate gene flow in natural populations. For example, the crustacean zooplankton are believed to be capable of widespread dispersal (Fryer, 1985) and many taxa have cosmopolitan distributions, but this belief remains to be confirmed by genetic studies.

The extent of individual movement between populations is difficult to estimate in most taxa and does not measure gene flow if immigrants have different reproductive success than residents. Thus, indirect quantifications of gene flow by examining the level of genetic differentiation among populations has been employed by a number of workers (Roberts and Hiorns, 1962; Nei and Imaizumi, 1966; Workman, 1968), especially since the development of rapid techniques

such as electrophoresis to identify genetic polymorphisms (Larsen et al., 1984; Pashley and Johnson, 1986; Liebherr, 1988; Wehrhahn and Powell, 1987; Singh and Rhomberg, 1987). However, the application of genetic models of differentiation relies on conditions that do not usually apply to many terrestrial taxa. For example, the island model (Wright, 1940) assumes there is symmetrical gene flow and that populations are discrete. Pond habitats and the zooplankton populations that inhabit them are discrete and most species are likely dispersed randomly among local populations (Talling, 1951; Jeffries, 1989) making them good model systems for studies on gene flow.

The long held view that many zooplankton species have cosmopolitan distributions has been recently challenged by systematists (Dumont, 1980; Frey, 1982) and their conclusions were consistent with expectations from studies which showed significant genetic divergence among local populations of several zooplankton taxa (Hebert, 1974; 1977; Hebert and Moran, 1980; King, 1977; Snell, 1979). These studies, however, have focussed on asexual taxa which may not be typical, because genetic differentiation could be extreme in such taxa if populations were founded from single individuals. No studies have examined genetic variation in the sexually reproducing freshwater zooplankters (Hedgecock et al., 1982) which also rely on passive dispersal. Many such taxa (e.g. cyclopoid copepods, or ostracodes) are apparently widespread in distribution suggesting their populations should be genetically uniform, at least locally.

This study has largely examined the extent and nature of biochemical genetic variation among populations of several copepod species. The genetic differentiation of populations of this and several other sexually reproducing zooplankton groups was used to evaluate the role of dispersal in the freshwater zooplankton.

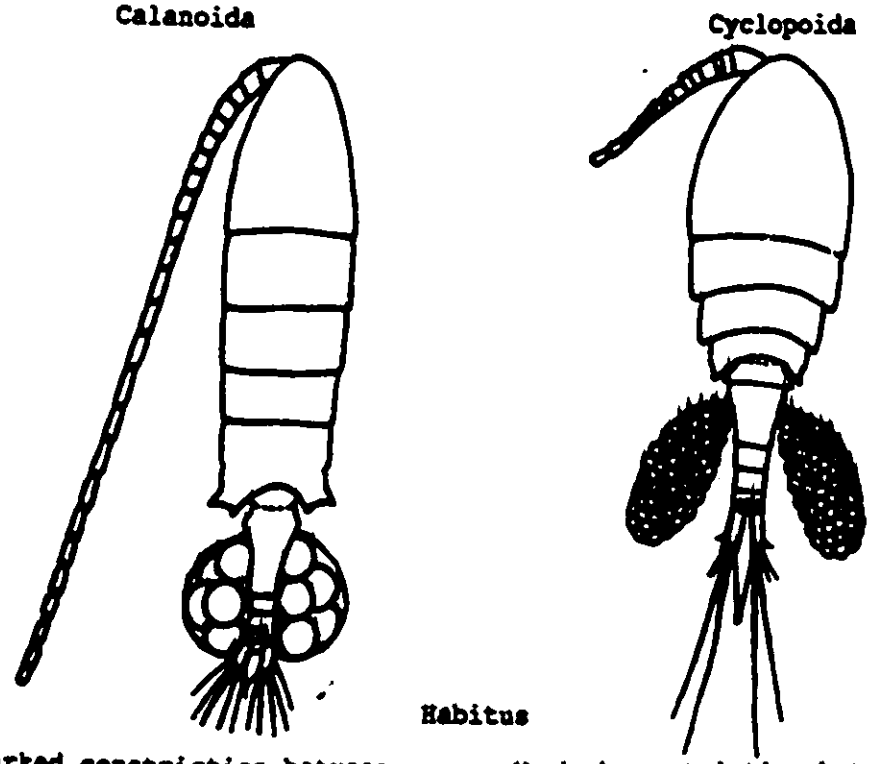
The Copepoda

The subclass Copepoda is a taxonomically diverse group of crustaceans consisting of nearly 10,000 species (Bowman and Abele, 1982; Marcotte, 1983; Schram, 1983). Pond habitats are occupied by members of three of the four free-living orders: Calanoida, Cyclopoida, Harpacticoida. Copepods are distinguished from other crustaceans primarily by the absence of a carapace and by their possession of a well developed cephalosome covering their five cephalic limbs (Schram, 1983). The calanoid body plan is similar to that of the cyclopoids (Figure 1-1), but is distinguished by the point of flexure between the metasomal and urosomal segments. The Harpacticoida can easily be distinguished from these two orders by their lack of any articulation between these two body sections. The work reported here includes studies on representative species from four families within the calanoids and cyclopoids (Table 1-1), which constitute the dominant pond fauna.

A unique anatomical terminology has developed for the Copepoda, which reflects the poor understanding of homologies with other Crustacea. Indeed, terminology has

Figure 1-1

Characteristics of two freeliving orders
(after Wilson and Yeatman 1959)



Calanoida	Cyclopoida
Marked constriction between somite of 5th leg and genital segment	Marked constriction between somites of 4th and 5th legs
1 egg sac, carried medially	2 egg sacs, carried laterally
Spermatophore elongate	Spermatophore kidney shaped
First Antennae	
Reach from near end of metasome to near end of caudal setae	Reach from proximal third of cephalic segment to end of metasome
23 to 25 segments; male right only geniculate (prehensile)	6 to 17 segments; male left and right geniculate
Leg 5	
Not vestigial; male asymmetrical	Vestigial; both sexes symmetrical

Table 1-1

Classification of the copepod taxa studied (according to Schram 1983)

Phylum: Crustacea Pennant, 1777

Class: Maxillopoda Dahl, 1956

Subclass: Copepoda Milne Edwards, 1840

Order: Calanoida Sars, 1903

Family: Centropagidae Giesbrecht, 1892

Genera & Species: Osphranticum labronectum S.A. Forbes, 1882
Limnocalanus johanseni Marsh, 1920

Family: Diaptomidae Baird, 1850

Genera & Species: Hesperodiaptomus eiseni (Lilljeborg, 1889)
H. arcticus (Marsh, 1920)
H. churchillensis n. sp.
H. nearcticus n. sp.
H. victoriaensis (Reed, 1958)
H. nevadensis (Light, 1938)
H. wilsonae (Reed, 1958)
Leptodiaptomus tyrrelli (Poppe, 1882)
L. pribilofensis (Juday and Muttkowski, 1915)
Arctodiaptomus bacillifer (Koelbel, 1884)
Aglaodiaptomus leptopus (S.A. Forbes, 1882)
A. stagnalis (S.A. Forbes, 1882)

Family: Temoridae Giesbrecht 1892

Genera & Species: Heterocope septentrionalis
Juday and Muttkowski, 1915
Eurytemora composita Keiser, 1929

Order: Cyclopoida Burmeister, 1834

Family: Cyclopidae Dana, 1853

Genera & Species: Acanthocyclops vernalis (Fisher, 1853)
A. robustus (Sars, 1863)
A. parcus (Herrick, 1884)
A. brevispinosus (Herrick, 1884)
A. americanus (Marsh, 1893
Diacyclops navus (Herrick, 1882)
Megacyclops latipes (Lowndes, 1927)

tended to differ from group to group within the Copepoda and no consensus has emerged to date (Dudley, 1986). The usage of Gurney (1931) for oral appendages and Sars (1901) for tagmata and swimming appendages has been adopted in this thesis because they are the most widely used in the two orders studied.

Copepods reproduce sexually and the sexes are dimorphic in all free-living taxa. During reproduction, males grasp females with their prehensile antennule(s) and deposit a sperm-filled spermatophore at the genital operculum. Eggs are fertilized internally and females of most species deposit their fertilized eggs into an external egg sac where some subsequent development takes place. Some species, such as Heterocope septentrionalis, do not carry fertilized eggs in an egg sac but rather fertilize and release the eggs individually. Development is immediate (subitaneous eggs) for all cyclopoid taxa, but in calanoids there is frequently a delay in complete embryogenesis (diapause) until a sequence of conditions stimulates final maturation and hatching. Leptodiaptomus tyrrelli (Hebert, 1985) and some other diaptomids (Hairston, 1987) have one to several clutches of subitaneous eggs prior to the production of diapause eggs. Because pond habitats typically either dry up and/or freeze solid in most years, the diapause stage is necessary for survival to subsequent seasons. Cyclopoids persist in the same habitats by arresting development in late pre-adult stages.

Postembryonic development is identical in these two

orders of Copepoda. There are 11 pre-adult stages in which the animal gets progressively larger and the limbs become fully developed. The eggs hatch into nauplii with only 3 pairs of appendages and progress through five naupliar molts (i.e. six stages). The full development of post mandibular appendages and the appearance of caudal rami marks the first to fifth copepodid stages with the final molt resulting in the mature adult (Schram, 1983).

There has been active interest in the Copepoda for more than 100 years (Milne-Edwards, 1840; Baird, 1850; deGuerne and Richard, 1889) but work on their genetics has been generally limited to cytological (Beerman, 1959, 1966, 1977; Columbera and Lazzaretto-Colombera, 1973), morphological (Battaglia, 1958; Volkmann-Rocco and Fava, 1969) and breeding studies (Battaglia and Volkmann-Rocco, 1973). One of the earliest studies employing electrophoresis (Manwell et al., 1967) determined that two morphologically similar marine species of Calanus produced different enzyme products. But further allozyme studies on copepods have been few (Bisol, 1976; Burton et al., 1979; Burton and Feldman, 1981; Nixon et al., 1981; Sevigny and Odense, 1983; Bucklin and Marcus, 1985) despite the recognized need for systematic research (von Vaupel Klein, 1984; Einsle, 1980, 1988) and the potential importance of copepods in extending the understanding of evolutionary mechanisms such as gene flow (Boileau and Hebert, 1988).

This study represents the single most extensive biochemical genetic survey on the Copepoda and is the only

study which has examined freshwater taxa. Although the study of the order Cyclopoida was confined to a few species in three genera, eight genera and all families of the North American calanoid fauna were included [Table 1-1; Schram (1983) excluded Pseudocalanidae classified by Wilson (1959)]. Calanoids dominate pond zooplankton communities in arctic North America and the nine species studied from this habitat (Limnocalanus johanseni, H. arcticus, H. victoriaensis, H. wilsonae, Leptodiaptomus tyrrelli, L. pribilofensis, Arctodiaptomus bacillifer, Heterocope septentrionalis and Eurytemora composita) represent 50% of the calanoid copepod fauna previously known in the arctic region and 91% of that known east of the Mackenzie River (Hebert and Hann, 1986).

Biochemical Genetic Variation

All studies in evolution involve implications concerning the extent and nature of genetic variation among individuals and populations of organisms. Until recently, most such studies employed morphological characters under either uncertain or complex genetic control. Although some cytological and biochemical studies were conducted (eg. Dobzhansky and Sturtevant, 1938; Landesteiner and Weiner, 1940), the techniques were slow, and extensive surveys of genetic variation were difficult. When it was recognized that electrophoresis of proteins produced variable patterns that could be interpreted as the products of alleles at single loci (Lewontin and Hubby, 1966; Harris, 1966),

evolutionary biologists began to employ the technique in broader surveys of genetic variation.

Enzyme electrophoresis separates enzyme products in a high voltage field (Gottleib, 1971) and detects about 25% of the actual variation in the DNA sequence which codes for the protein (Nei, 1987). Mendelian genetic control of enzyme patterns has been established in many organisms by mating studies (Richardson et al., 1986) but can also be inferred from phenotype frequencies and patterns where confirmation by mating is impractical (Selander et al., 1970). For example, if the electromorphs conform to the patterns expected from the known quaternary structure of the enzyme in other taxa (Harris and Hopkinson, 1976; Richardson et al., 1986) and the proportions of the phenotypes conform to those expected at Hardy-Weinberg equilibrium, then it is appropriate to infer that the patterns are controlled by allelic diversity at a single locus. Such isozymic patterns which are inferred to be the products of alternate alleles at a single locus are referred to as allozymes.

Electrophoretic methods are now routine (Shaw and Prasad, 1970; Harris and Hopkinson, 1976; Richardson et al., 1986) and it is now possible to visualize the products of many enzyme loci from single individuals. The methods utilized in the present studies are those of Hebert and Beaton (1989) which employ cellulose acetate gels as an electrophoretic medium.

Dispersal

Where genetic variation exists in populations of a species, gene frequencies within the populations can diverge. Population genetics theory predicts that selectively neutral genetic variation will differentiate in discrete populations (Wright, 1943) because gene frequencies will fluctuate randomly in each population. The magnitude of the gene frequency fluctuations will be controlled by the size of the populations and the equilibrium variation in frequencies by the number of genes the populations exchange. Increased gene frequency differentiation is countered by gene exchange which brings genes, possibly lost or in low frequency in one population, from others where they may predominate. Gene frequencies will fluctuate more in smaller populations which are completely isolated than in larger populations which exchange migrants. Wright (1943) further demonstrated that a stationary state can be reached in these populations where the variation in gene frequencies among populations does not change (although the frequencies within individual populations do) because populations are independently becoming more different due to random fluctuations and simultaneously becoming more similar due to the receipt of genes from other populations.

Empirical attempts to verify this theory have been equivocal although, where parameters such as population sizes and age are known the theory is testable. Considerable effort has focussed on the aspect of whether allozymes satisfy the conditions of selective neutrality

(reviews by Scharloo et al., 1977; Koehn, 1978; Koehn et al., 1983). Others have attempted to determine whether observed differentiation of allozymes is compatible with known (or more often approximated) levels of dispersal (Zera, 1981; Varvio-Aho, 1983; Leibherr, 1988).

On a broader geographic scale, theory also predicts that taxa which found new populations from ancestral sources can lose genetic variability (Nei et al., 1975) in relation to the number of colonists. Organisms, which have colonized habitats by repeated dispersal events after continental deglaciation, might be expected to reflect this loss of genetic variability with particular clarity. Therefore, populations in the vicinity of glacial refugia should be genetically more variable than those in more recently deglaciated habitats.

The main goal of the present study was to examine the extent of allozyme variation in freshwater copepods with particular emphasis on the nature of variation in the arctic Canadian fauna. The extensive allozyme studies served as a basis for detailed analyses of the patterns of variation within species. The nature of variation within species can be used to estimate gene flow and to reconstruct the manner in which arctic Canada was recolonized after deglaciation. As well, aliozyme data provide a clear indication of reproductive isolation in taxa and permit the clarification of species boundaries.

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Chapter II

Characteristics of Population Genetic Variation in Freshwater Copepods

Introduction

Although members of the Copepoda comprise a dominant component of the fauna in most aquatic habitats, biochemical genetic work on this group has been extremely limited (Hedgecock et al., 1982). Prior allozyme studies have examined genetic variation in only one or two species (Manwell et al., 1967; Burton et al. 1979; Burton and Feldman 1981; Nixon et al., 1983; Bucklin and Marcus, 1985; Sevigny and Odense 1983) and all have dealt with marine taxa. Copepod species in freshwater ponds occupy habitats which are discrete, yet genetic exchange is presumed to be high due to the widespread distributions of many taxa. If this is true, populations in such habitats are expected to be genetically uniform. No genetic work has confirmed this, but studies (Burton et al. 1979; Burton and Feldman 1981) have shown moderate differentiation in a marine tidepool copepod despite the potential for dispersal of their pelagic larvae.

Enzyme electrophoresis was employed in the present study to examine genetic variation in 16 calanoid and 8 cyclopoid taxa from pond habitats. The aim of the study was to determine the amount of genetic variation in these copepods and the genetic and ecological factors that influence it. Genotypic frequencies were ascertained in single populations to determine if the individuals mate

randomly. Although genetic characteristics such as sex determination, and karyotypes are unknown for most copepod taxa, especially the calanoids (Colombera and Lazzaretto-Colombera, 1978), sex-linked enzymes are known among the Crustacea (Shaklee, 1983; Havel et al., 1990). For this reason, genotype frequency differences among sexes and autosomal linkage equilibrium were examined also. A compilation of the variation among 24 freshwater species permitted a comparison of freshwater Copepoda with both their marine relatives and other crustaceans.

A detailed analysis of microspatial variation of gene frequencies in the three dominant copepod species at Churchill, Manitoba was also undertaken. These species show differential distribution patterns, which suggest that their dispersal capabilities may differ. Heterocope septentrionalis, a large predator, inhabits virtually all ponds in the tundra habitat as does a smaller diaptomid Leptodiaptomus tyrrelli. However, the latter species is also found ubiquitously in another local habitat type, the ponds found in glacial scours of bedrock outcrops, while the former is not. A third species Hesperodiaptomus victoriaensis is found contagiously distributed in both habitat types. These observations suggested that populations of these copepods may show variable levels of genetic differentiation. Specifically, L. tyrrelli and Heterocope ought to be less differentiated than H. victoriaensis in the tundra habitat because of their ubiquity in the habitat. Leptodiaptomus tyrrelli as well

should be less differentiated on rock bluffs than its diaptomid counterpart, for the same reason.

Temporal and environmental factors which could influence genetic variation were also investigated in detail. Undetected temporal fluctuations in gene frequencies could confuse the interpretation of the significance of spatial differences. Previous work on both copepods (Burton and Feldman, 1983) and cladocerans (Weider and Hebert, 1987) has revealed that physical parameters such as ionic concentrations influenced the distributions of some genotypes and suggested the value of a search for similar correlations in the present study.

Material and Methods

Collections

Copepod populations belonging to 24 taxa in two major orders (Cyclopoida, Calanoida) of the subclass were collected from 31 sites (Appendix I) in North America and Britain. The identities of North American cyclopoid taxa were determined using descriptions by Sars (1918), Herrick (1884), and Marsh (1893), for *Acanthocyclops* spp. as well as Yeatman (1959) for all others. Cyclopoid specimens from Britain were identified using Kiefer (1976) and subsequently confirmed by Dr. G. Fryer. The North American calanoids were identified using Wilson (1959). Two previously undescribed species of calanoids (*Hesperodiaptomus nearcticus*, *H. churchillensis*; see Chapter 5) were also studied. Some species were collected at several localities,

while others were obtained from single populations. Animals were removed from the populations by hand or throw net and kept alive at 4°C until electrophoresis.

Electrophoresis

Usually 24 or more individuals were homogenized separately and electrophoresed on cellulose acetate gels (Helena Laboratories). Gels were run for 10-20 minutes in a Tris Glycine (pH 8.5) or a CAEA (pH 7.2) buffer according to the methods of Hebert and Beaton (1989). Enzymes which were stained and could be scored regularly included: aldehyde oxidase (AO), amylase (AMY), arginine phosphokinase (APK), fumarase (FUM), glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), mannose-phosphate isomerase (MPI), peptidase (PEP-C using phenlyalanylproline and PEP-D using leucylglycine as dipeptide substrates), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), triose-phosphate isomerase (TPI) and xanthine dehydrogenase (XDH). All enzymes were not necessarily scored in all taxa.

Genetic variation in the Copepoda

In order to determine whether calanoid copepod populations were randomly mating within ponds and to infer the allelic nature of the enzyme patterns observed, the phenotypic frequencies at all polymorphic enzymes were compared with the Hardy-Weinberg expectations (HWE) by chi-square. Only samples with more than 25 individuals were

tested and where an expected phenotype class was less than 1 this class was pooled. Gene frequencies for all loci were determined by direct count.

Sex in the calanoid copepods is known to be determined chromosomally, although cytogenetic studies have been carried out on only a few taxa (Colombera and Lazarretto-Colombera, 1978). In some species males are heterogametic while in others they are homogametic. In order to determine whether any of the loci were sex-linked, sexes were separated prior to electrophoresis for each polymorphic locus in eight calanoid species. The presence of heterozygotes in both sexes confirmed autosomal inheritance, while their absence in one sex indicated sex-linkage.

The genotypes of individual animals from many calanoid populations were ascertained at multiple loci. In order to determine whether there was linkage disequilibrium between loci, a test of independence (Sokal and Rohlf, 1981) was conducted on all pairwise combinations of loci where at least 20 individuals were sampled. Genotypes at enzyme loci that had more than two alleles segregating were pooled to produce classes representing the segregation of the most common allele and all others. This eliminated rare genotypic classes that would inflate the G-statistic where the expectation for the class was less than five.

The extent of genetic variation in each of 24 species was quantified using four parameters: percent of loci polymorphic (two criteria, P_{99} and P_{95}, where the frequency of the most common allele was not greater than 0.99 and 0.95

respectively); number of alleles per locus (A, no minimum frequency criterion); heterozygosity (H_e, Nei, 1977; unbiased where samples <24). The distribution of H_e estimates among loci were determined for each species and unweighted means for Calanoida and Cyclopoida were calculated.

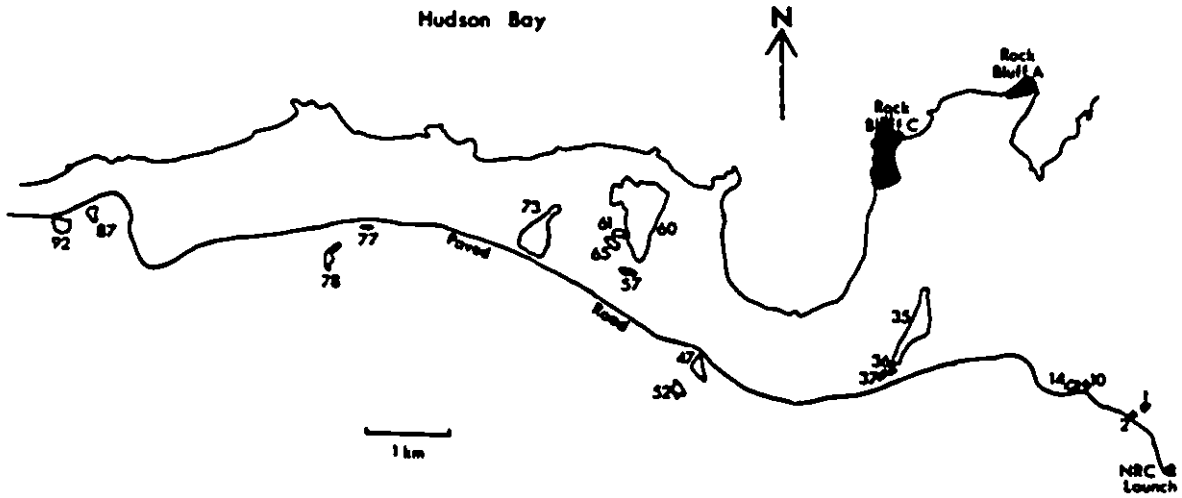
Detailed Analyses of Arctic Calanoids

An intensive study of three species (Heterocope septentrionalis, Leptodiaptomus tyrrelli and Hesperodiaptomus victoriaensis) at Churchill, Manitoba was undertaken between 1983 and 1986 in order to examine spatial and temporal gene frequencies among populations. Gene frequencies from 25 populations were analysed at five different polymorphic loci to determine if any significant shifts occurred in genotype frequencies at these loci over three years. Heterogeneity of genotype frequencies were tested by the G-test of independence (Sokal and Rohlf, 1981).

Gene frequencies at 2-3 polymorphic loci were analysed in 58 populations of the same three species collected over 15 km coastline at this site in two habitat types, tundra and rock bluff (Figure 2-1). Spatial variation of gene frequencies was examined by hierarchical F-statistics (Nei, 1977) for multiple allele systems and the significance of the genetic differences was evaluated by X^2 (Workman and Niswander, 1970).

Figure 2-1

Locations of ponds along coastline of Hudson Bay east of Churchill, Manitoba where detailed studies were undertaken.



Three levels of gene frequency differentiation were examined: demes within habitats (F_{DH}); demes within the site (F_{DS}); and habitats within site (F_{HS}). $F_{DH}=(H_H-H_D)/H_H$ and $F_{DS}=(H_S-H_D)/H_S$ where H_D, H_H and H_S are the average heterozygosity of loci within demes, among habitats and over the site, respectively. These hierarchical measures are related to the average inbreeding coefficients over loci (Nei, 1977), within demes (F_{ID}) and within the site (F_{IS}) by the equation,

$$1-F_{IS} = (1-F_{ID})(1-F_{DH})(1-F_{HS}).$$

Correlations between allele frequencies at four polymorphic enzymes and four physical parameters (light absorbance @ 350nm, pH, conductivity and alkalinity), which were measured independently (Hebert and Billington, in prep.), were examined to determine if there were any detectable relationships between the allele frequencies and these environmental factors. Correlation coefficients were calculated between the arcsine square root transformed frequency of the most common allele and the respective parameters.

Results

Levels of Variation

Polymorphisms were detected in every species examined (Table 2-1) and loci such as APK, MPI, PGI, PGM and XDH were polymorphic in more than 60% of the species. The quaternary structures of enzymes were the same as those reported in

Table 2-1

Enzyme descriptions and characteristics in cyclopoid (Cyc.) and calanoid (Cal.) Copepoda. Frequency is the percent of species studied (number in parentheses) which were polymorphic (no criterion).

Enzyme/E.C.#	Locus designation	Quaternary structure	# of loci	(%) polymorphic Cyc.	Cal
Aldehyde oxidase 1.2.3.1	AO	Dimer	1-3	37.5 (8)	13.3 (15)
Amylase 3.2.1.1	AMY	Monomer	1->1	--- (1)	31.2 (16)
Arginine phosphokinase 2.7.3.3	APK	Monomer	5-6	12.5 (8)	62.5 (16)
Fumarase 4.2.1.2	FUM	Tetramer?	1	12.5 (8)	53.3 (15)
Glutamate oxaloacetate transaminase 2.6.1.1	GOT	Dimer	2	50.0 (8)	56.2 (16)
Isocitrate dehydrogenase 1.1.1.4	IDH	Monomer	2,3?	25.0 (8)	70.0 (10)
Lactate dehydrogenase 1.1.1.27	LDH	Tetramer?	1	12.5 (8)	0.0 (9)
Malate dehydrogenase 1.1.1.37	MDH	Dimer	2	50.0 (8)	56.2 (16)
Malic enzyme 1.1.1.40	ME	Tetramer	2	12.5 (8)	0.0 (15)
Mannosephosphate isomerase 5.3.1.9	MPI	Monomer	1	80.0 (8)	71.4 (14)
Peptidase 3.4.11	PEP	Dimer	1-3	--- (1)	58.3 (12)
Phosphoglucose isomerase 5.3.1.9	PGI	Dimer	1-2	62.5 (8)	62.5 (16)
Phospho- glucomutase 2.7.5.1	PGM	Monomer	1	100 (8)	100 (16)
Triosephosphate isomerase 5.3.1.1	TPI	Dimer	1	--- (1)	33.3 (3)
Xanthine dehydrogenase 1.2.1.37	XDH	Dimer	1	--- (0)	77.8 (9)

studies of other organisms (Harris and Hopkinson, 1976; Richardson et al., 1986) with one exception. The monomeric structure of both IDH loci (confirmed by the presence of two banded heterozygotes in seven species of calanoids) differs from the dimeric structure typical of vertebrates. Some frequently polymorphic enzymes were represented by multiple loci in most taxa; in particular APK was always represented by at least 4 loci of varying stain intensity.

Tests for goodness of fit to HWE were conducted on 436 samples from five calanoid taxa at three sites (Boileau and Hebert 1988a;b; Appendix II). Genotypic frequencies in most populations conformed to HWE, however, deviations did occur in 39 (9%) tests. In most instances where deviations occurred, the populations did not deviate at more than one locus in the same individuals. As well, the electromorphs observed always corresponded to the homozygote and heterozygote enzyme phenotypes expected if the loci were controlled by a single locus with segregating alleles. Three enzymes (AMY, APK and PEP) produced complex multiple zones in several species. Phenotypic frequencies at some of these zones neither conformed to HWE nor produced electromorphs that could be explained as simple genetic polymorphisms and these were not reported.

No evidence for sex linkage was obtained in eight different calanoid taxa as heterozygotes were observed (Table 2-2) in both sexes at all polymorphic loci. Pairwise comparisons of seven polymorphic loci were made 25 times in Heterocope septentrionalis and 16 times combined in the

Table 2-2

Sex linkage in eight species of Calanoida. Hs-*Heterocope septentrionalis*; Ec-*Eurytemora composita*; Hv-*Hesperodiaptomus victoriaensis*; Ha-*H. arcticus*; Hc-*H. churchillensis*; Hn-*H. nearcticus*; Lt-*Leptodiaptomus tyrrelli*; Al-*Aglaodiaptomus leptopus*.

Enzyme Locus	Family: Temoridae		Diaptomidae					
	Hs	Ec	Hv	Ha	Hc	Hn	Lt	Al
PGI	X		X		X			
PGM	X			X	X			X
AMY-3	X							
MPI		X		X	X	X	X	X
MDH	X		X		X			
AO-3	X		--	--	--	--	--	--
IDH-1							X	
APK-4		X	X		X	X		X
APK-5			--	--	--	--	X	--
FUM	X				X	X		
GOT-1			X					
GOT-2	X							
PEP-C	X					X		
XDH			X	X	X	X		

X=heterozygotes observed in both sexes
--=locus not present

Hesperodiaptomus arcticus and H. churchillensis for six loci. None of the tests revealed any evidence of non-random association among genotypes at different loci (Appendix III).

The extent of genetic variation within species of Copepoda was similar in the two orders studied with none of the measures of genetic variation differing significantly between these groups. For example, heterozygosity in 16 species of Calanoida averaged 0.093±.0004 (Table 2-3) and 0.078±.0015 (t=1.36, P>.05) in 8 species of Cyclopoida (Table 2-4). The heterozygosities of the polymorphic loci were comparably distributed (Figure 2-2) with highly variable loci (H_e 0.6-0.8) in both orders.

Detailed Analyses of Arctic Calanoids

Significant temporal shifts in gene frequencies were observed in only 4 of 32 tests conducted over four years (Table 2-5). All but one of the shifts occurred in three year comparisons and the separate two year comparisons made on these populations were all non significant. No significant shifts were observed in the nine tests of H. septentrionalis populations.

The gene frequencies of all three species were significantly variable (Table 2-6, 2-7, 2-8) within all habitats. Heterocope was the most differentiated species within the tundra habitat (F_{DH}=.100; Table 2-8) while the other two species were similar, and much less so (F_{DH}=.039, H. victoriaensis; F_{DH}=.032, L. tyrrelli). The mean level of

Table 2-3

Biochemical genetic variation of allozyme loci in 16 species of Calanoida. (s=# of populations studied; L=# of loci examined; n=# animals sampled/locus; P_{95}=% polymorphic loci, 95% criterion; P_{99}=% polymorphic loci, 99% criterion; H_e=HWE expected heterozygosity; A=# of alleles/locus)

Species	s	L	n	P_{99}	P_{95}	A	H_e
Calanoida							
Centropagidae							
Osphranticum labronectum	1	15	23	40.0	33.3	1.47	.120
Limnocalanus johanseni	1	16	24	12.5	12.5	1.12	.032
Diaptomidae							
Hesperodiaptomus eiseni	15	15	474	26.6	20.0	1.53	.043
H. arcticus	6	21	208	23.8	19.0	1.28	.063
H. nearcticus	17	21	601	30.2	25.5	1.38	.075
H. churchillensis	6	21	248	36.5	28.5	1.57	.123
H. victoriaensis	10	21	397	49.6	44.3	1.60	.138
H. nevadensis	1	16	27	31.2	31.2	1.50	.123
H. wilsonae	1	15	36	26.7	26.7	1.27	.065
Leptodiaptomus tyrrelli	4	21	98	60.9	53.2	2.0	.215
L. pribilofensis	1	18	24	38.9	38.9	1.94	.177
Arctodiaptomus bacillifer	1	14	21	35.7	35.7	1.50	.110
Aglaodiaptomus leptopus	4	21	132	30.2	25.4	1.36	.080
A. stagnalis	1	17	37	35.3	17.6	1.35	.073
Temoridae							
Heterocope septentrionalis	32	23	1141	36.1	32.3	1.51	.108
Eurytemora composita	1	17	26	23.5	23.5	1.29	.086
Means (SE)[1]				33.6 (2.8)	29.2 (2.6)	1.48 (.06)	.098 (.012)

1. P_{99}, P_{95} and H_e arcsine transformed for analysis.

Table 2-4

Biochemical genetic variation of allozyme loci in 8 species of Cyclopoida. (s=# of populations studied; L=# of loci examined; n=# animals sampled/locus; P_{95}=% polymorphic loci, 95% criterion; P_{99}=% polymorphic loci, 99% criterion; H_e=HWE expected heterozygosity; A=# of alleles/locus)

Species	s	L	n	P_{99}	P_{95}	A	H_e
Cyclopoida							
Cyclopidae							
Acanthocyclops vernalis UK	3	16	29	14.6	14.6	1.21	.052
A. robustus UK	1	16	30	6.2	6.2	1.13	.032
A. parcus	5	17	55	37.6	35.3	1.48	.183
A. brevispinosus	1	17	30	41.2	35.3	1.71	.128
A. americanus	1	17	13	17.6	11.8	1.24	.052
A. robustus	1	17	33	23.5	23.5	1.53	.126
Diacyclops navus	1	20	5	25.0	25.0	1.35	.110
Megacyclops latipes	4	24	38	12.5	12.5	1.12	.013
Means (SE)[1]				21.2 (4.3)	19.5 (3.9)	1.35 (.07)	.078 (.021)

1. P_{99}, P_{95} and H_e arcsine transformed for analysis.

Figure 2-2

Distribution of heterozygosities in 16 species of Calanoida (a) and 8 species of Cyclopoida (b).

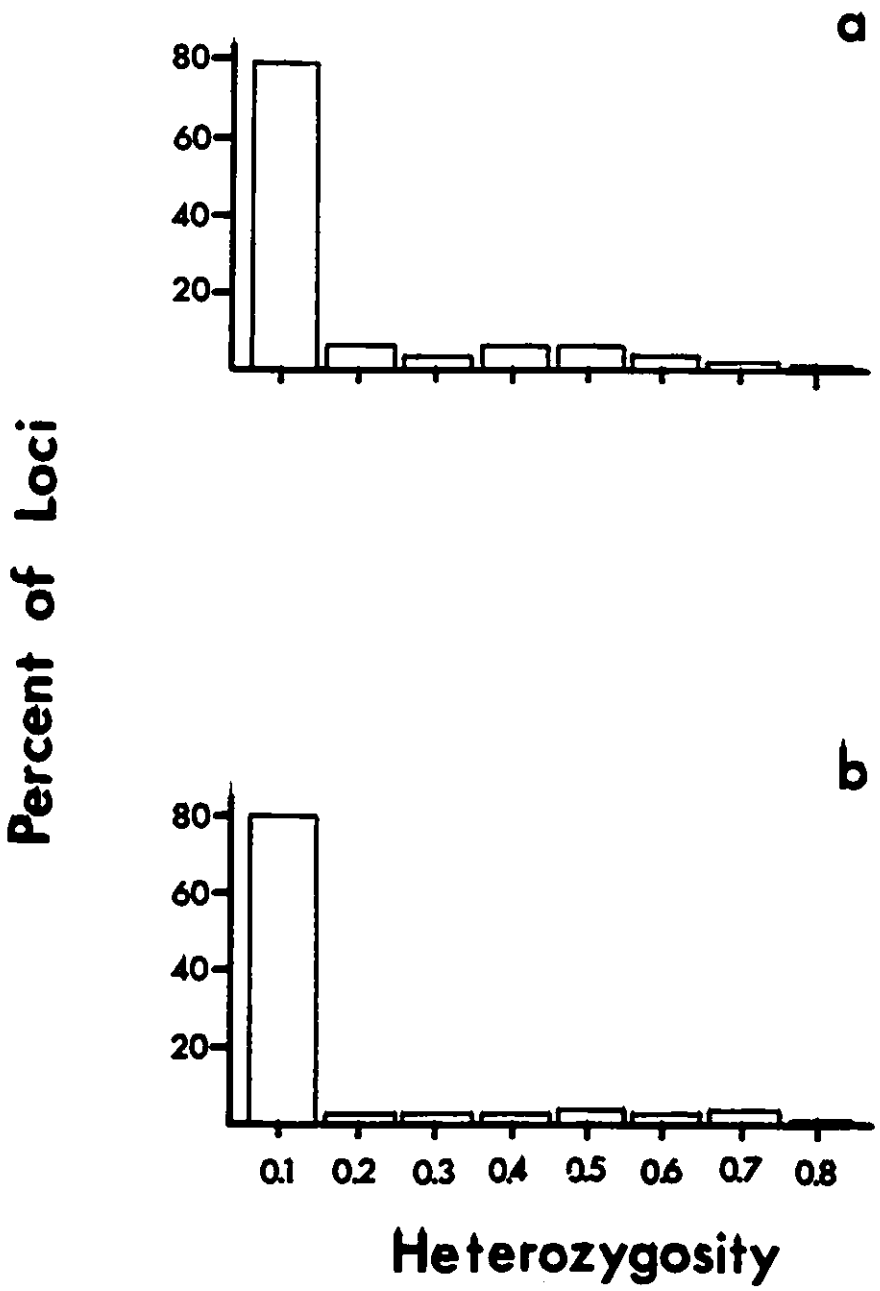


Table 2-5

Temporal variation of phenotype frequencies at allozyme loci in 3 species of calanoids over 4 years at Churchill, Manitoba. Xs signify years from which variation was tested. G_{adj}=G statistic adjusted using Williams correction (Sokal and Rohlf, 1981).

Species/ /Pond	Locus	Year '83	'84	'85	'86	G_{adj}	df
Heterocope septentrionalis							
T1	PGI	X	X	X		7.13	4
T2	PGI		X	X		0.15	2
T10	PGI	X	X	X		6.19	4
T14	PGI		X	X		4.92	2
T37	PGI		X	X		0.61	2
T47	PGI		X	X		2.68	2
T76	PGI	X	X			2.28	2
T79	PGI	X	X			1.42	2
T99	PGI	X	X			1.88	2
Hesperodiaptomus victoriaensis							
A3	PGI			X	X	1.79	2
A5	PGM		X	X		3.52	5
	PGI		X	X		0.74	2
	GOT		X		X	4.82	2
A6	PGM		X	X	X	15.60	10
	PGI		X	X	X	8.66	4
	GOT		X		X	0.34	2
A13	PGI		X	X		4.66	2
	GOT		X	X		0.78	2
A21	PGM		X	X		6.90	5
	PGI		X	X		1.35	2
T37	PGM		X	X	X	29.61*	10
	PGI		X	X		0.75	2
	GOT		X	X	X	12.01*	4
Leptodiaptomus tyrrelli							
A60	PGM		X	X		2.07	4
C20	PGM		X	X		8.44	5
T1	PGM		X	X		1.97	4
T14	PGM		X	X		9.06	5
T37	PGM		X	X		3.53	5
T73	PGM		X	X		1.62	5
T76	PGM	X	X			12.99*	5
T77	PGM	X	X	X		10.98	10
T78	PGM	X	X	X		30.01*	10

*-G significant, P<.05

Table 2-6

Spatial distribution of gene frequencies at two enzyme loci in *L. tyrrelli* from the tundra and bluff habitats.

Habitat/ Population	Gene Frequencies PGI 1	PGI 2	PGM 1	PGM 2	PGM 3
Tundra					
T1	.48	.52	.04	.65	.31
T2	.46	.54	.04	.59	.37
T10	.43	.57	.07	.68	.25
T14	.30	.70	.06	.62	.32
T35	.53	.47	.18	.59	.23
T37	.40	.60	.12	.67	.21
T73	.53	.47	.14	.46	.40
T77	.59	.41	.08	.57	.35
T78	.62	.38	.10	.43	.47
T87	.60	.40	.20	.48	.32
Sample	494		678		
F_{DH}	.037		.028		
X^2(df)	36.56(9)*		37.97(18)*		
Rock Bluff A					
A3	.47	.53	.23	.57	.20
A9	.20	.80	.28	.70	.02
A13	.27	.73	.16	.60	.24
A17	.32	.68	.10	.70	.20
A21	.33	.67	.23	.53	.24
A22	.26	.74	.26	.58	.16
A25	.14	.86	.21	.64	.15
A38	.31	.69	.31	.39	.30
A38a	.36	.64	.19	.54	.27
A60	.24	.76	.39	.59	.02
Sample	481		544		
F_{DH}	.037		.037		
X^2(df)	35.59(9)*		40.26(18)*		
Rock Bluff C					
C12	.72	.28	.17	.25	.58
C13	.76	.24	.13	.19	.68
C20	.70	.30	.16	.42	.42
C27	.68	.32	.21	.43	.36
C45	.69	.31	.40	.38	.22
C53	.88	.12	.23	.43	.34
C84	.55	.45	.20	.45	.35
C90	.44	.56	.07	.44	.49
C113	.81	.19	.14	.61	.25
C152	.62	.38	.13	.06	.81
Sample	477		546		
F_{DH}	.067		.099		
X^2(df)	63.92(9)*		108.1(18)*		

* X^2 values significant P<.05

Table 2-7

Spatial distribution of gene frequencies at 3 enzyme loci in H. victoriaensis from tundra and Bluff A.

	Gene Frequencies						
	PGI		PGM			GOT	
Population	1	2	1	2	3	1	2
Tundra							
T36	.14	.86	.28	.43	.29	.44	.56
T37	.09	.91	.19	.80	.01	.43	.57
T57	.19	.81	.32	.46	.22	.32	.68
T60	.25	.75	.25	.40	.35	.30	.70
T61	.19	.81	.32	.37	.31	.39	.61
T65	.17	.83	.38	.37	.25	.38	.62
T92	.73	.27	.19	.56	.25	.12	.88
Sample	389		316			347	
F_{DH}	.062		.056			.075	
X^2	48.24(6)*		35.39(12)*			52.05(6)*	
Rock Bluff A							
A3	.20	.80	.20	.56	.24	.30	.70
A4	.31	.69	.22	.63	.15	.21	.79
A5	.25	.75	.25	.62	.13	.35	.65
A6	.14	.86	.20	.56	.24	.36	.64
A9	.17	.83	.13	.51	.36	.25	.75
A13	.26	.74	.01	.84	.15	.51	.49
A20	.02	.98	.18	.34	.48		1.0
A21	.07	.93	.18	.57	.25	.21	.79
A22	.12	.88	.31	.64	.05	.32	.68
A25	.04	.96	.33	.54	.13	.22	.78
A44	.09	.91	.17	.57	.26	.22	.78
Sample	859		773			607	
F_{DH}	.213		.056			.046	
X^2	365.9(10)*		86.58(20)*			55.84(10)*	

* X^2 significant P<.05

Table 2-8

Spatial distribution of gene frequencies at 2 enzyme loci in Heterocope septentrionalis from the tundra habitat.

	Gene Frequencies							
	PGI		PGM				AMY	
Population	1	2	1	2	3	4	1	2
T1	.29	.71	.31	.41	.25	.03	.88	.12
T2	.36	.64	.41	.41	.15	.03	.85	.15
T10	.39	.61	.72	.22	.04	.02	.79	.21
T14	.40	.60	.54	.38	.07	.01	.91	.09
T35	---	---	.60	.33	.07		---	---
T37	.34	.66	.48	.50	.01	.01	.81	.19
T47	.32	.68	.90	.10			.55	.45
T52	.22	.78	.59	.24	.17		.60	.40
T77		1.0	.28	.42	.30		.82	.18
T92	.51	.49	.29	.59	.12		.53	.47
Sample	774		471				409	
F_{DH}	.083		.107				.104	
X^2(df)	128.5(8)*		100.8(30)*				85.1(8)*	

* X^2 significant P<.05

differentiation in bluff populations of L. tyrrelli (mean F_{DH}=.062) was similar to the value for H. victoriaensis bluff populations (Bluff A=.064). However, gene frequencies in populations of these two species in the same habitat (Rock Bluff A) showed higher differentiation in H.victoriaensis.

There was no tendency for the populations within bluff habitats to be less differentiated due to their closer proximity than tundra populations. In fact, H. victoriaensis and L. tyrrelli, both found in the two habitats were less differentiated over 10 km of tundra habitat than they were in less than 0.5 km of Bluff A. All species had similar values of F_{DS}, differentiation of demes over the entire site, but most noticeable was the contribution of the differences in mean gene frequencies between habitats in L. tyrrelli to its overall gene frequency heterogeneity.

The frequencies of PGI^1 and PGM^3 alleles (Figure 2-3) in L. tyrrelli from the Bluff C habitat were markedly different from their frequencies in the remaining habitats at the site ($F_{HS[PGM]}$=.044, X^2[4 d.f.]=311.2; $F_{HS[PGI]}$=.107, X^2[2 d.f.]=310.7). These persistent frequency differences within habitats contributed most of the heterogeneity ($\overline{F}_{HS}$=.072; Table 2-9) at the site. Gene frequency differences between habitats in H. victoriaensis were much less pronounced ($\overline{F}_{HS}$=.007).

Three species were examined for correlations between

Figure 2-3

Gene frequencies at two polymorphic loci (PGM & PGI) in L. tyrrelli from Churchill, Manitoba. Gene frequencies for Rock Bluffs A & C are unweighted mean frequencies of 10 ponds in each habitat. Size of each pie section is proportional to gene frequency.

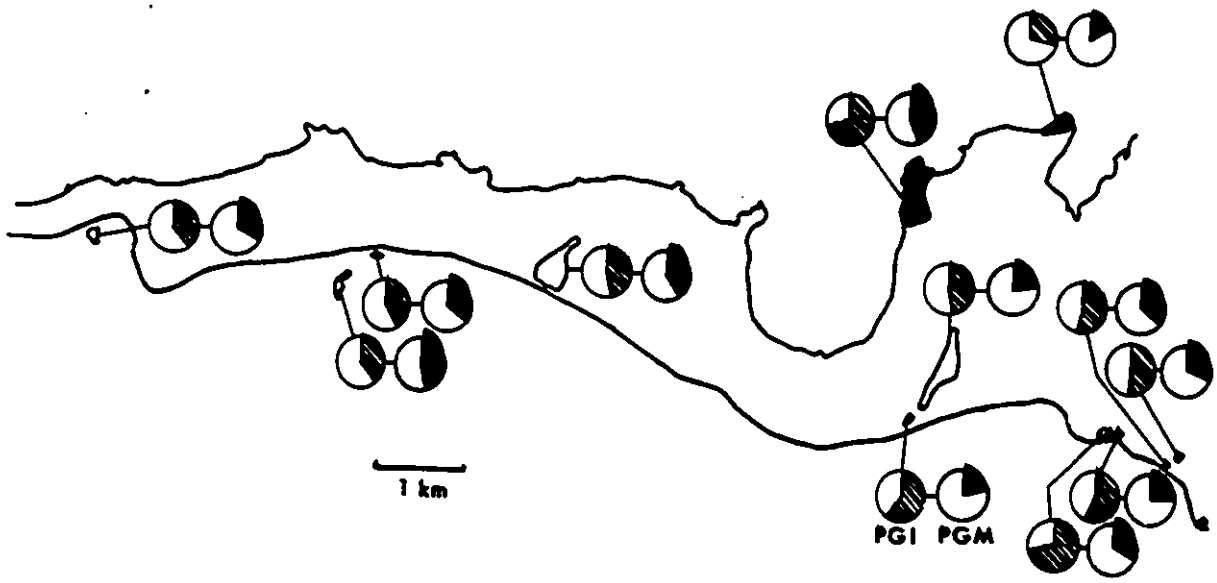


Table 2-9

Hierachical F-statistics for three species of calanoid copepods at Churchill, Manitoba. Levels of the hierarchy are demes (D) within habitats (H; Rock Bluffs and Tundra) and the entire site (S). See text for details of calculations.

Species	Level	$\overline{F}_{DH}$	$\overline{F}_{HS}$	$\overline{F}_{DS}$
L. tyrrelli	Tundra	.032		
	Rock Bluff A	.038		
	Rock Bluff C	.085		
	Bluff means	.062		
	Site	.050	.072	.118
H. victoriaensis	Tundra	.039		
	Rock Bluff A	.064		
	Site	.075	.007	.081
H. septentrionalis	Tundra	.100	---	.100

gene frequencies and four physical parameters: absorbance @ 350nm, alkalinity (m mol L^{-1}), conductivity (uS cm^{-1}) and pH. Only three correlations were significant of 29 examined and only two of these accounted for more than 25% of the variation in the arcsine square-root transformed gene frequencies (Table 2-10). Plots of the significant relationships reveal that similar gene frequencies occur over wide ranges of physical measures (Figures 2-4, 2-5).

Discussion

This study has examined a significant proportion (~18%) of the freshwater copepod fauna from North America and demonstrates their considerable variability. As many as five to ten polymorphic enzyme systems can be routinely expected in modest electrophoretic surveys of species within the group. Most polymorphisms appear to be controlled by alternate alleles at single Mendelian loci with the genotypic frequencies approximating Hardy-Weinberg expectations. Although genotypic frequencies in some populations differed from expectations, deviations are expected by chance and the pooling criterion used (expected frequency <1) tends to give inflated X^2 values.

No difference was detected in the levels of genetic variation among the calanoids and cyclopoids and Figure 2-6 illustrates the similar distributions of two correlated parameters (P_{99} and H_e) in these two taxa. It has been suggested that allozyme variation is not evenly distributed over taxonomic groups in the Crustacea (Hedgecock et al.

Table 2-10

Coefficients of determination (r^2) between gene frequencies (angular transformation of most frequent allele) and physical parameters (sample sizes in parentheses). Parameters decribed in text.

	L. tyrrelli		*H. victoriaensis*			*H. septentrionalis*		
	PGM	PGI	PGM	PGI	GOT	PGM	PGI	AMY
ABS	.03	.20	.02	.00	.00	.12	.42*	.02
	(38)	(30)	(23)	(21)	(18)	(10)	(17)	(10)
ALK	.00	.04	.01	.05	.06	---	---	---
	(18)	(14)	(7)	(8)	(7)	---	---	---
COND	.04	.20	.03	.05	.05	.22	.00	.14
	(44)	(30)	(24)	(24)	(20)	(10)	(17)	(10)
pH	.16*	.38*	.04	.00	.00	.07	.00	.04
	(50)	(30)	(24)	(24)	(20)	(10)	(17)	(10)

* correlation coefficient (r) significant P<.01

Figure 2-4

Relationships between L. tyrrelli PGM^2 and PGI^1 gene frequencies and pH.

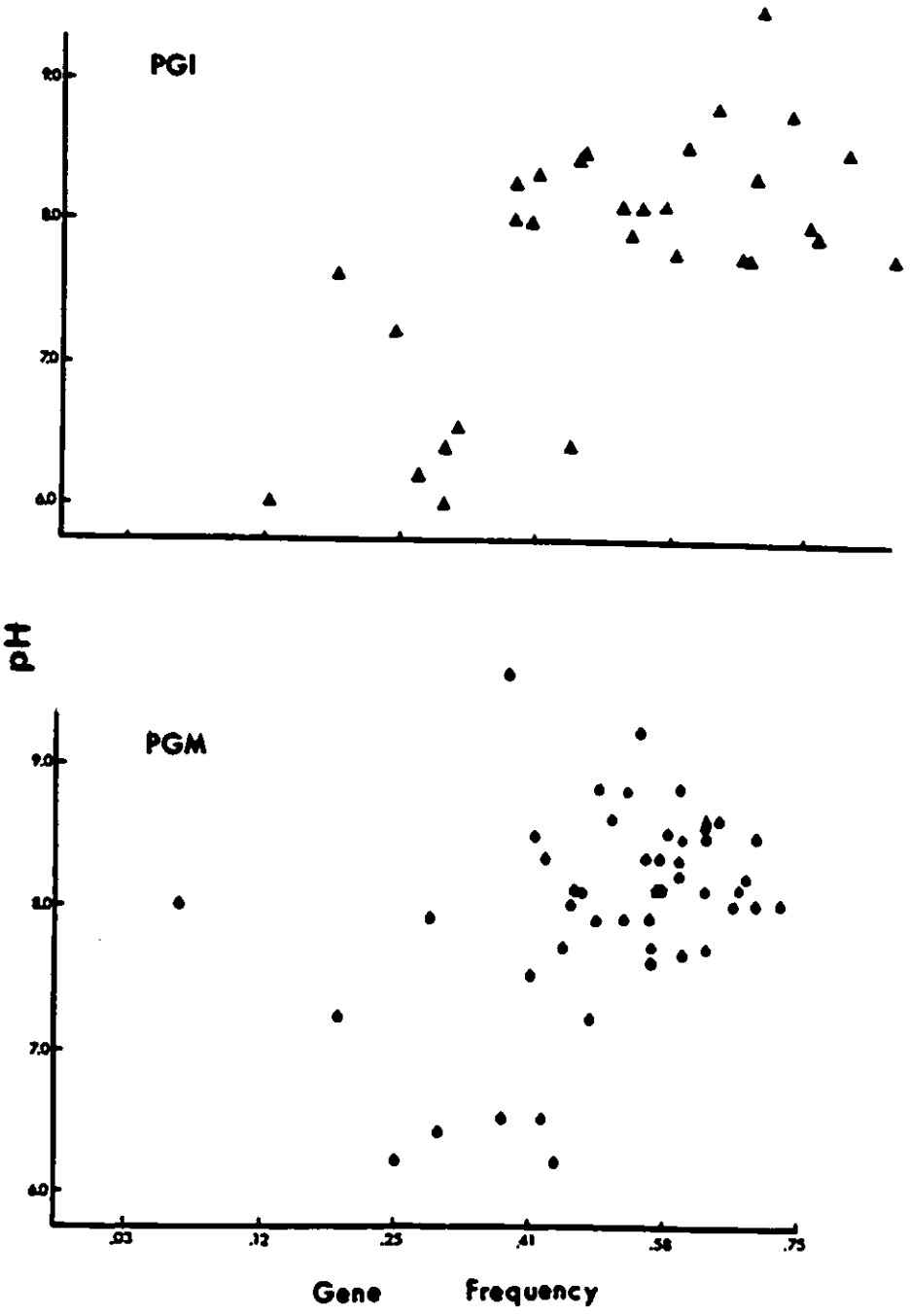


Figure 2-5

Relationship between H. septentrionalis PGI^2 gene frequencies and absorbance at 350nm.

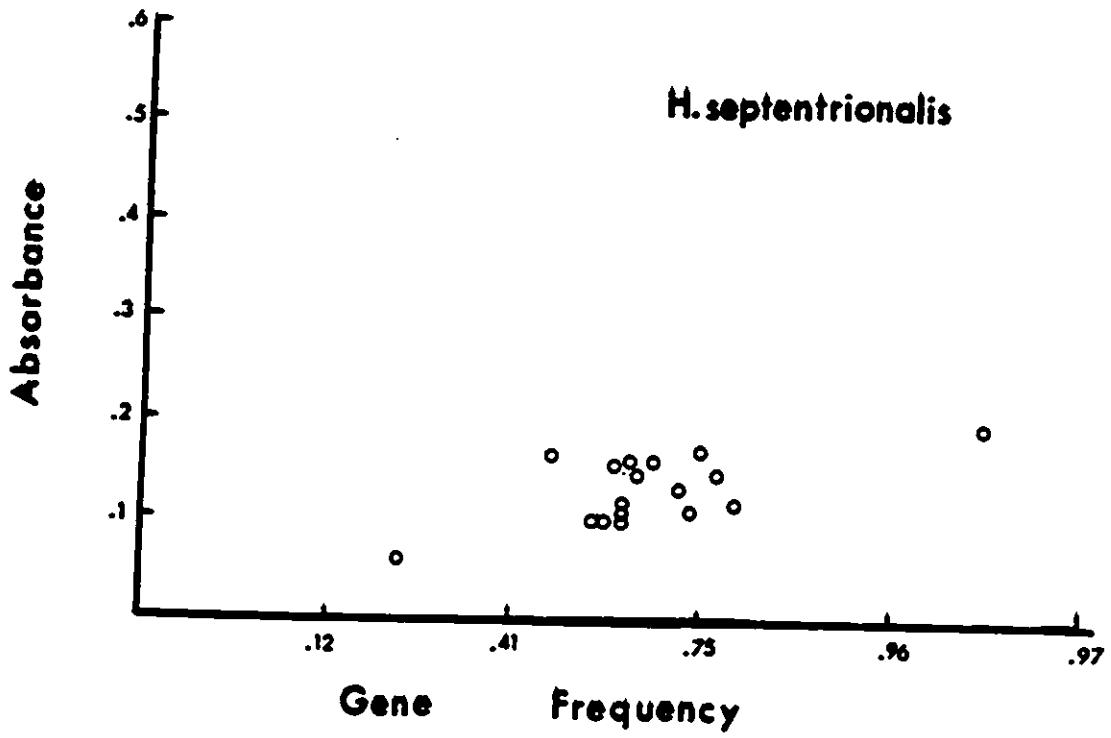
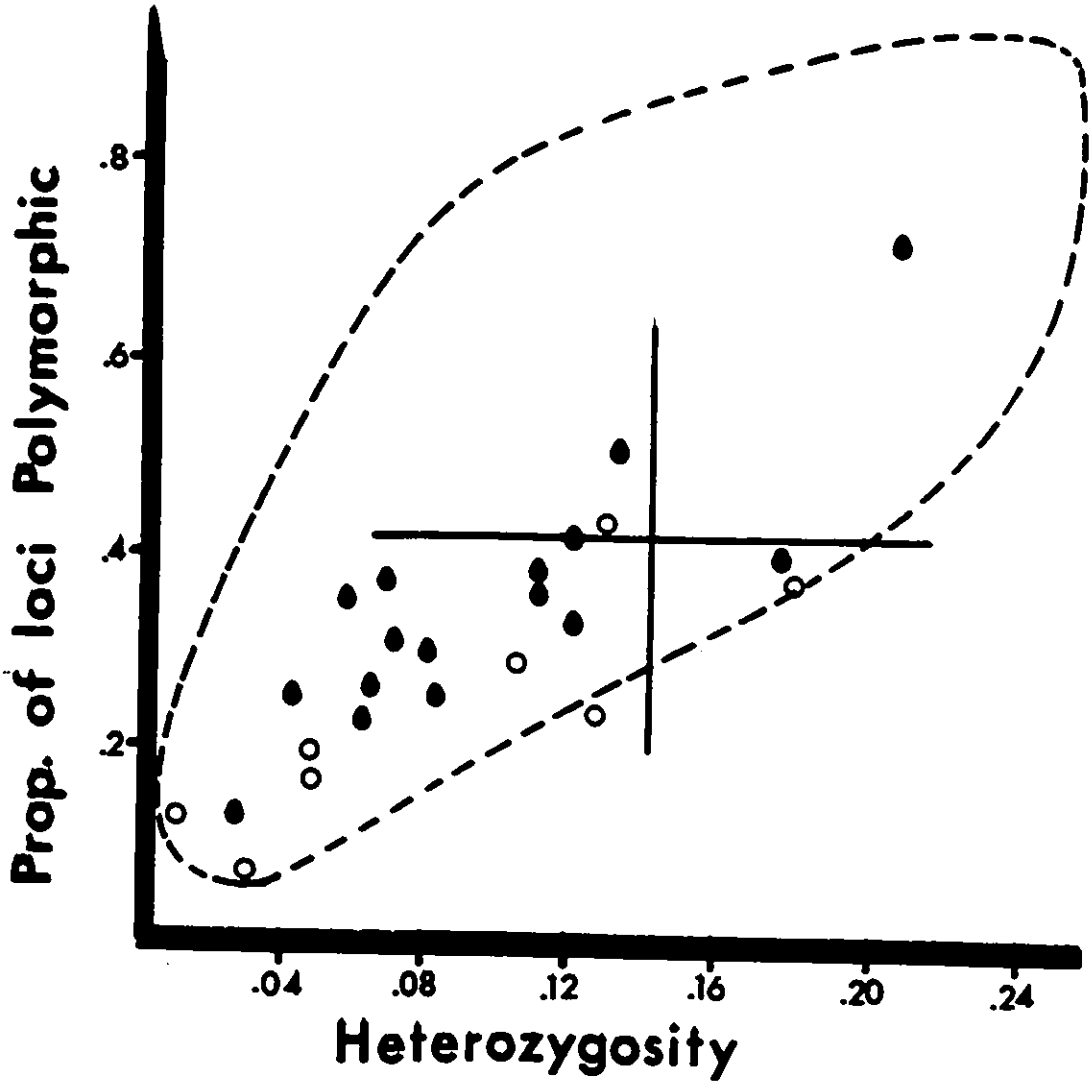


Figure 2-6

Average heterozygosity (H_e) and proportion of polymorphic loci (P_{99}; see text for definition of these parameters). Open circles-Cyclopoida, closed circles-Calanoida; this study. Solid cross (+)-mean and one standard deviation of 5 marine Copepoda, dashed line-range of Crustacea; Hedgecock et al., (1982).



1982) and the copepods were cited among the four highly variable taxa. However, genetic variation in the freshwater copepods spans most of the range observed in the Crustacea in general, and the estimates for species were more variable than those for marine copepods (Figure 2-6). Although these authors recognized that most groups had been inadequately sampled, a general trend for higher heterozygosity with small body size was also noted. No correlation existed between body size and H_e (R^2=0.07) or any of the other measures of genetic variation in the present study. This demonstrates the danger of characterizing the level of variation in a higher taxon by examining only a few species and indicates that there remains a need to understand the factors which affect genetic variation.

The detailed studies at Churchill revealed that populations of freshwater copepods are not genetically uniform, even in localized habitats. Temporal variation of gene frequencies was usually insignificant, but its occurrence and nondirectional nature suggests some mechanisms that should be investigated. Three of the four significant temporal changes occurred in large tundra ponds where population sizes are considerable (Boileau and Hebert, 1988a) ruling out fluctuations of frequencies due to genetic drift. The temporal differences could reflect the sampling of different panmictic subpopulations within these large ponds, but this too seems unlikely, because persistent winds circulate these shallow isothermal water bodies. Spatial variation within ponds, however, was not investigated and

cannot be ruled out as an important agent in explaining apparent temporal shifts.

Gene Frequency Differentiation and Dispersal of Taxa

The present study has shown that substantial gene frequency differences are common among copepod populations in localized habitats. Significant gene frequency differentiation among populations is, perhaps, expected in copepods (Burton, 1986), but extreme genetic differentiation of populations only several meters apart has only been observed in parthenogenetic zooplankton (Hebert, 1974). Differentiation of gene frequencies in this case was attributed to their ability to found populations from single individuals. Sexually reproducing species must necessarily found populations from more individuals, but the patchy distribution of H. victoriaensis hints that this result might have been anticipated. However, the broad distribution of this species as well as Heterocope, L. tyrrelli and many other copepod taxa suggests that a paradox might exist between dispersal and genetic differentiation in these organisms.

Leptodiaptomus tyrrelli occurs broadly in montane and arctic habitats across North America (Wilson, 1959) but allozyme work (Boileau and Hebert, 1988b Appendix II) confirmed some previous doubts about the validity of this widespread distribution. Two genetically distinct species were confirmed (L. tyrrelli and L. pribilofensis) each with a non-overlapping portion of the total distribution. It is

possible that many other North American taxa, which have such reported widespread distributions, are actually cryptic species complexes. Only comprehensive genetic surveys that define copepod species more accurately will clarify such misleading biogeographical information.

The variation in the extent of genetic differentiation observed among conspecific populations of different species was inconsistent with predictions based on their local distributional patterns. Specifically, H. victoriaensis was less differentiated among the Churchill populations than the other two species despite its considerably more restricted and patchy distribution. In fact, H. septentrionalis was more differentiated within the tundra habitat than either H. victoriaensis or L. tyrrelli. These results suggest that the level of genetic differentiation among populations need not reflect dispersal ability.

Further evidence is found in partitioned genetic differentiation statistics. The significant differentiation of average gene frequencies in the three habitats where L. tyrrelli occurred contributed most of the differentiation to the site. This suggests that populations may become founded in clusters from a single nucleus population and that entire habitats may reflect the genetic characteristics of that nucleus. If so, then present populations reflect this founder effect either because dispersers are genetically ineffective, or the populations have not had sufficient time to exchange genes and equilibrate local gene frequencies.

Glaciers receded from the Churchill area about 7000

years ago (Teller et al., 1983), but the specific habitats near the Hudson Bay coastline did not emerge from the sea until 2000 to 3000 years ago. The organisms that inhabit the ponds likely did not become established immediately afterwards, but nevertheless, it is not known how many generations of continuous dispersal would be required for the groups of populations to achieve their equilibrium level of gene frequency differentiation. The use of genetic differentiation alone, to predict dispersal in such organisms appears to be unwarranted and possibly misleading.

In summary, freshwater copepod taxa possess a considerable amount of genetic variation. Genotypic frequencies at variable loci tend to be in Hardy-Weinberg equilibrium and to show little temporal variation. Significant microspatial variation in gene frequencies among conspecific populations is common and is not apparently linked to environmental heterogeneity, but rather appears to reflect the residual impact of founder effects.

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Chapter III

Non-Equilibrium Gene Frequency Divergence: Persistent Founder Effects In Natural Populations

Introduction

Theoretical studies show that gene flow among populations has an important impact on the evolutionary potential of a species (Slatkin, 1987), but the direct determination of gene exchange among natural populations is impossible for most taxa. In principle, however, the level of gene flow can be inferred from the amount of gene frequency divergence among populations. Wright (1943, 1951) established a simple relationship between gene flow and the amount of gene frequency divergence of neutral alleles among populations. Employing an island model of population structure, where organisms occur in discrete demes with a uniform probability of exchanging migrants, Wright (1943) demonstrated that gene frequency differentiation (F_{ST}) reaches a non-zero equilibrium whose magnitude is linked in a simple fashion to the number of migrants. More recently, Slatkin (1985) has developed a second indirect method of using gene frequency information to infer gene flow. His simulation studies established that, at equilibrium, there is a precise relationship between the average frequency of alleles found in only one population ("private alleles"; Neel, 1973) and gene flow among demes.

These relationships have been employed in a number of studies to estimate levels of gene flow among populations

over broad geographic areas (Dobzhansky and Queal, 1938a,b; Roberts and Hiorns, 1962; Nei and Imaizumi, 1966; Workman, 1968; Larsen et al., 1984; Pashley and Johnson, 1986; Waples, 1987; Wehrhahn and Powell, 1987; Singh and Rhomberg, 1987; Liebherr, 1988). The use of island models to estimate gene flow on a macrogeographic scale is probably inappropriate because dispersal among demes is not likely to be either equal or symmetric. Few workers have attempted to examine gene pool variation at multiple enzyme loci and estimate gene flow in more local situations (Fleischer, 1983; Caccone, 1985; Caccone and Sbordoni 1987; Hebert and Payne, 1985; Sweeney et al., 1986; Ward et al., 1987; Boileau and Hebert, 1988), in part due to the paucity of taxa which exist in discrete demes.

Organisms in pond habitats have a population structure like that considered in Wright's island model; demes are discrete and gene flow among them may well be equally probable at least in physically compact regions (Talling, 1951; Jeffries, 1989). Prior studies on the genetics of the invertebrate fauna of ponds have concentrated on cladoceran crustaceans, which typically reproduce by cyclic parthenogenesis. These studies have shown large gene frequency differences among local populations of these taxa (Hebert, 1974; Hebert and Moran, 1980; Schwartz and Hebert, 1987). The fragmented gene pools of cladocerans are thought to arise as a consequence of founder effects associated with a reliance on passive dispersal and the ability of single parthenogenetic individuals to found populations. There

remains a need to establish the generality of such gene pool fragmentation in bisexual pond dwelling organisms.

This study involved the analysis of genetic differentiation among populations of 18 invertebrate taxa at two sites in arctic Canada. Both sites have a high density of ponds and both areas are undergoing active isostatic rebound so that the habitats under study are less than 3000 years old (Andrews, 1970; Klassen, 1983). The dominant taxa in ponds at each site are entomostracan crustaceans and turbellarian flatworms, both of which re-establish populations each year from resting eggs. Passive dispersal of the resting eggs, supposedly by birds and wind, is required to found new populations. The insect fauna of these pond habitats is impoverished, but collembolans were abundant at one site. These organisms are capable of active movement, but, as they lack flight, one might anticipate a low exchange of individuals among ponds similar to the dispersal of other flightless insects (Zera, 1981). The present study found significant gene frequency divergence in all taxa and no association between the extent of differentiation and dispersal potential. As a result, factors other than low dispersal rates that might account for such differentiation were considered.

When populations are founded by small numbers of individuals, one anticipates some level of genetic divergence associated with colonization events. These initial differences can subsequently be eroded by gene exchange among populations until an equilibrium is reached. There

have been few efforts to examine the likelihood that gene frequencies in natural populations are at equilibrium. Theoretical treatments (Latter, 1973; Nei et al., 1977) have examined the simultaneous subdivision of a single large population into a finite number of identical smaller panmictic subpopulations. Under these conditions genetic differentiation increased in early generations and then declined gradually toward zero. Similarly, Allendorf and Phelps (1981) and Slatkin (1985) used computer simulations to establish that the time required to reach equilibrium in subdivided populations is dependent upon m, the proportion of each subpopulation which disperses each generation and not mN_e, the absolute number of migrants exchanged. Allendorf and Phelps (1981) concluded that significant gene frequency divergence among fish populations need not imply low levels of gene exchange. The present study aimed specifically to examine the likelihood that the gene frequency distributions of aquatic invertebrates represent equilibrium conditions.

This study used a computer simulation to consider the events associated with the founding of populations and the subsequent decay of gene frequency differences. The model examined the length of time required for migration to erode gene frequency differences which arose during colonization. Unlike previous analytical approaches (Latter, 1973; Nei et al., 1977), this model assumed that genetic divergence existed among subpopulations before dispersers were exchanged. The model indicated that the length of time

required to erode gene frequency differences varied with migration rate, and showed that founder effects can persist for thousands of generations even in the face of substantial migration.

Material and Methods

Empirical Studies

Gene frequency divergence of pond invertebrates was studied at two sites in the Canadian Arctic: 8-31 populations from 11 taxa at Igloolik, N.W.T. (69^{o}N; arctic) and 10-37 populations from 10 taxa at Churchill, Manitoba (59^{o}N; subarctic). Both sites have abundant pond habitats (>5 km^{-2}). Ponds chosen for study were ordinarily separated by at least 10 meters from their nearest neighbour. Analysis was restricted to species which reproduce sexually or by cyclic parthenogenesis.

Collections at Igloolik were made between July 30 and August 15 of 1986 and 1987 from ponds broadly distributed over the western half of the island. Eight crustacean taxa were included in the survey including: two anostracans - *Branchinecta paludosa* and *Artemiopsis stefanssoni*; three copepods - *Hesperodiaptomus eiseni*, *Eurytemora composita* and *Cyclops canadensis*; two cladocerans - *Chydorus sphaericus* and *Eurycercus glacialis*; and one notostracan - *Lepidurus arcticus*. In addition *Mesostoma arctica*, a rhabdocoel turbellarian, and two collembolans (*Isotomurus ciliatus* and *Podura aquatica*) were analyzed.

Collections at Churchill were made between June 15 and

August 31, 1984-1988. Nine crustacean species were analyzed including: one anostracan- Branchinecta paludosa; one ostracode- Cyprinotus glaucus; three cladocerans - Ceriodaphnia reticulata, Eurycercus glacialis and Simocephalus vetulus; and four copepods - Hesperodiaptomus arcticus, Hesperodiaptomus victoriaensis, Heterocope septentrionalis and Leptodiaptomus tyrrelli. The turbellarian, Mesostoma arctica, was also analyzed.

Freshwater ponds at Churchill occur in two distinct habitats; quartzite rock bluffs adjacent to Hudson Bay and low-lying tundra (Weider and Hebert, 1987). Sampling of the former habitats concentrated on two rock bluffs (A and C), which lie approximately 1500 m apart, and which have been the subject of several earlier studies (Hebert and Payne, 1985; Weider and Hebert, 1987; Boileau and Hebert, 1988). Rock bluffs are compact and ponds on a single bluff may be less than 10 m apart, whereas tundra ponds are typically separated by hundreds of meters or more. In the present study an effort was made to include populations from both habitat types, although some species (e.g., H. septentrionalis) occur in only a single habitat type (tundra).

Twenty-four individuals from each of 2-3 populations per taxon at both sites were examined for allozyme variation in 2-20 enzyme systems. Variation was classified as a genetic polymorphism only when the phenotypes of putative heterozygotes were congruent with those expected on the basis of the usual quaternary structure of the enzyme in question (Harris and Hopkinson, 1977; Richardson et al.,

1986). If this preliminary survey identified polymorphism at one or more loci, gene frequencies at these loci were determined by analyzing 24-48 individuals from each of 8-37 populations. Loci which were polymorphic in one or more taxa included: amylase (AMY), aldehyde oxidase (AO), glutamate oxaloacetate transaminase (GOT), mannose-6-phosphate isomerase (MPI), malate dehydrogenase (MDH), phosphoglucose isomerase (PGI) phosphoglucomutase (PGM) and triosephosphate isomerase (TPI) and 2 dipeptidases (leucylglycine peptidase, PEP-C; phenylalanylproline peptidase, PEP-D). Individuals of smaller species (e.g. Chydorus sphaericus) whose body lengths were less than 0.5 mm, only stained reliably for enzymes with high activity, and were examined for variation at fewer loci than were taxa with a larger body size. Animals of all species were electrophoresed within 24 hours of collection using Titan III cellulose acetate gels (Easteal and Boussy, 1987). Gels were run at 200 volts for 15 minutes in a Tris Glycine buffer (pH 8.5). Details of electrophoretic procedures are available (Hebert and Beaton, 1989).

Gene frequencies at all polymorphic loci were determined by direct count, and the extent of gene frequency differentiation (F_{ST}) among populations of each taxon was determined using the methods of Nei (1977) for each locus. The significance of the differences among populations at each locus was assessed by the χ^2 statistic ($\chi^2 = 2N F_{ST} [k-1]$, k = number of alleles) where F_{ST} was corrected for sample sizes (Workman and Niswander, 1970).

The overall unbiased estimate of genetic differentiation was determined using gene diversities corrected for the sample size (harmonic means) and number of populations sampled (Nei, 1986).

Relationship between Dispersal Capacity and Estimated Dispersal Rate

Qualitative evaluations of the dispersal capacities of taxa were done using dispersal enhancement criteria. Species which had the potential for active, free living dispersal were scored highest. Passive dispersal of adults or resting stages was assumed to be enhanced by: 1) morphological accesscries which permit water or wind flotation, 2) pigmentation, which affords visibility to predators, 3) resistance to digestion, 4) persistence of eggs for several years before hatching, and 5) formation of clutches which permits many eggs to move in one dispersal event. In some cases information for a specific taxon was unavailable, but was inferred from studies on closely related species. Species were ranked according to their total accumulation of enhancement characters.

A quantitative estimate of the number of dispersers exchanged per generation ($\underline{mN}_e$) was made using the overall F_{ST} estimated for each species in the relation,

$$\underline{mN}_e = \frac{1 - \underline{F}_{ST}}{4\underline{F}_{ST}} .$$

Most populations from a site generally shared the same

allelic arrays so the private allele model of Slatkin (1985) could not be used. The qualitative and quantitative estimates of dispersal were compared using Kendall's coefficient of rank correlation (Sokal and Rohlf, 1981).

Simulation Studies

A computer simulation (Appendix IV) was employed to ascertain the length of time required to decay genetic differences established at the time of colonization of habitats. The model began with a source population which was polymorphic for two alleles in equal frequency and 25 subpopulations were founded by $\underline{K}$=1, 5 or 10 individuals using a Monte Carlo simulation. Subpopulations were allowed to expand to 10^4, 10^5 or 10^6 individuals in one generation followed by the exchange of dispersers. The number of dispersers per generation ($\underline{mN_e}$=1, 5, 10) equalled the number of founders ($\underline{K}$) because there is likely a link between the number of colonists and subsequent dispersal. Cyclically parthenogenetic taxa can found new populations from one genetically effective individual so this extreme limitation was employed in the simulations. The subpopulations received immigrants from the disperser pool by a Monte Carlo simulation which selected from the entire substructured population before reproduction, for each of 2000 generations. New gene frequencies were calculated by direct count and the F_{ST} was calculated every generation. Each generation was discrete and disperser individuals were chosen from the total population. The effect of exchanging

1, 5 and 10 dispersers per generation was simulated for all three subpopulation sizes and each simulation was replicated 100 times.

The models were intentionally simplified by excluding drift and mutation in individual populations. Both forces would act to slow the erosion of gene frequency differences, and hence, further extend the time required to decay the gene frequency divergence which arises during population founding.

Results

Empirical Studies

Among the 10 species analyzed at Churchill and 11 at Igloolik, only three species were present at both sites. *Eurycercus glacialis* was invariant at both localities in 17 enzyme systems examined (PGI, PGM, AO, GOT, AMY, MPI, MDH, ME, FUM, PEP-C & D, G3PDH, 6PGDH and ACON) representing 18 loci. *Branchinecta paludosa* was invariant at Igloolik, but polymorphic at Churchill in 2 (AMY and PGI, Table 3-1) of the 9 enzymes which could be resolved (monomorphic systems included APK, GOT, MDH, ME, FUM, PEP-D & C). The third species, *M. arctica*, showed variation (Hebert and Payne, 1985) at both sites, with three polymorphic loci at Igloolik (MPI, PEP-D and PGM) and two polymorphic loci (MPI and PGM) at Churchill. Polymorphisms were identified in all other species except *L. arcticus* at Igloolik (Beaton and Hebert, 1988), and *C. reticulata* (AMY, AO, GOT, PGI and PGM) at Churchill. Specific details of loci studied and levels of

Table 3-1

The significance of gene frequency differences at polymorphic loci examined in populations of 15 pond invertebrate species from Igloolik and Churchill. n = harmonic mean of sample sizes; s = number of populations studied; F_{ST} = locus specific gene differentiation; * = F_{ST} significantly different from 0, P χ^2<.001; $\bar{F}_{ST}$ = unbiased gene differentiation (Nei's G_{ST}).

Site/Species	n	s	Locus	F_{ST}	$\bar{F}_{ST}$
Igloolik					
A. stefanssoni	42.6	15	GOT	.022*	.075
			PEP	.186*	
			PGM	.030*	
C. sphaericus	51.9	12	PGI	.071*	.067
C. canadensis	47.4	10	MPI	.064*	.053
			PGM	.048*	
H. eiseni	46.0	8	AMY	.094*	.149
			MDH	.129*	
			PEP	.223*	
			TPI	.038*	
E. composita	50.2	9	PGI	.120*	.132
I. ciliatus	51.6	10	PGI	.065*	.045
			PGM	.034*	
P. aquatica	52.8	11	PGI	.076*	.020
			PGM	.021*	
M. arctica	41.8	31	MPI	.032*	.033
			PEP	.053*	
			PGM	.034*	

Table 3-1 continued

Churchill					
B. paludosa	43.3	37	AMY PGI	.132* .350*	.360
C. glaucus	118.8	21	FUM GOT MPI PGI	.210* .183* .124* .141*	.171
S. vetulus	25.0	23	AMY AO PGI	.176* .298* .064*	.254
H. arcticus	42.8	9	MPI PEP-C	.122* .043*	.106
H. victoriaensis	51.4	18	GOT PGI PGM	.068* .148* .062*	.058
L. tyrrelli	50.0	30	PGI PGM	.145* .096*	.115
H. septentrionalis	38.5	10	AMY PGI PGM	.104* .083* .107*	.093
M. arctica	45.6	32	MPI PGM	.189* .113*	.122

polymorphism for other taxa are reported elsewhere (Boileau and Hebert, 1988; Havel et al., 1990).

Eight taxa with at least one polymorpic locus were studied at Igloolik in 8-31 populations and the same number of taxa were studied at Churchill in 10-37 populations (Table 3-1). Genotypic frequencies at polymorphic loci were generally in good agreement with Hardy-Weinberg expectations. There were only 28 (5.4%) significant ($P<.05$) deviations out of 519 cases tested. This result provided an indirect confirmation of the genetic basis of the observed electrophoretic patterns.

Gene frequencies were significantly different among conspecific populations at all loci and all species (Table 3-1). Unbiased estimates of F_{ST} ranged from 0.020 to 0.360. Taxa at Igloolik showed less gene frequency differentiation (mean F_{ST} for all species=0.072) than those at Churchill (mean F_{ST}=0.160), and the only species studied at both sites (M. arctica) was also much less differentiated at Igloolik (F_{ST}=0.033) than Churchill (F_{ST}=0.122).

Dispersal Capacities

Vagility indices ranged from 1 to 6 (Table 3-2) with highest scores for the two collembolans (I. ciliatus; P. aquaticus), which can disperse actively. All other taxa rely on passive dispersal, and all but one species (C. canadensis) had some characters which enhance dispersal. Details of Cyclops canadensis biology are unknown due to its recent discovery (Einsle, 1988), but cyclopoid copepods typically do

Table 3-2

Characteristics of dispersal capacities in 15 aquatic[a] invertebrate taxa at two study sites in northern Canada. Passive egg enhancement categories include; A=morphological assists, B=digestion resistance, C=persistance in egg banks >1y., D=eggs laid and carried in clutches, E=pigmentation in eggs or adults. mN_e calculated from unbiased F_{ST} in Table 3-1.

Species	Active Dispersal	Passive Adults	Passive Egg Enhancements A	B	C	D	E	Vagility Score	mN_e
L. ciliatus	likely	---						6	12.3
P. aquatica	likely	---						6	5.3
C. glaucus	none	likely		X	X	X	X	5	1.2
S. vetulus	none	none	X	X	X		X	4	0.7
C. sphaericus	none	none	X	X	X		X	4	3.5
M. arctica	none	none		X	X	X	X	4	4.6[b]
H. eisenі	none	none		X	X	X	X	4	1.4
H. arcticus	none	none		X	X	X	X	4	2.1
H. victoriaensis	none	none		X	X	X	X	4	4.1
L. tyrrelli	none	none		X	X	X	X	4	1.9
E. composita	none	none		X	X	X	X	4	1.6
E. paludosa	none	none		X	X	X		3	0.4
A. stefanssoni	none	none		X	X	X		3	3.1
H. septentrionalis	none	none		X	X		X	3	2.4
C. canadensis	none	likely[c]			none			1	4.5

a Collembolans are not truly aquatic.
b Mean of 2 estimates from Igloolik (7.3) and Churchill (1.8) used in overall comparison only.
c Only likely means of dispersal.

not have diapausing eggs. Diapause is accomplished through encystment of subadult stages which survive dessication (Cole, 1953; Fryer and Smyly, 1954) but do not likely survive transit through animal digestive tracts. On the other hand the survival of anostracan, cladoceran, copepod, notostracan, and ostracode eggs and even ostracode adults through animal digestive tracts is known (Lowndes, 1930; Proctor, 1964; Proctor and Malone, 1965; Proctor et al. 1967; Mellors, 1975). Turbellarian eggs probably survive as well. Unlike other copepods, females of Heterocope septentrionalis do not carry their clutches of fertilized eggs in a sac (Hebert, 1985). Instead, eggs are released immediately after fertilization, and hence dispersal is limited to individual eggs.

The estimates of mN_e ranged from less than 1 in two cases, to 12.2 individuals per generation (Table 3-2). The ranked scores of vagility and mN_e were not significantly correlated when all taxa were considered (r_s=0.402, P<.05). However, because there was an obvious difference in mean F_{ST} between the two sites, separate ranked comparisons were conducted for each site. Neither of these comparisons was significant ($r_{s\ Igloolik}$=0.134, P>.05; $r_{s\ Churchill}$=0.356, P>.05).

Simulation Studies

Gene frequency differences due to colonization in the simulations were congruent with those expected from sampling theory (F_{ST}=s-1/2Ks, where s=# of populations sampled). A

significant decline in F_{ST} was noted (Figure 3-1) within a few hundred generations when population sizes were small (10^4). However, when population sizes were larger (10^5, 10^6) divergence showed little decay over a 2000 generation interval even under considerable migration pressure (mN_e=10). Using the simulation results, it was possible to calculate the number of generations required to decay half of the initial gene frequency divergence. The half-life (H) of F_{ST} increased by an order of magnitude when either the number of dispersers was decreased or the population size was increased by an order of magnitude (Table 3-3). Thus, the half-life of F_{ST} was inversely related to the percent of the population that dispersed:

$$H \simeq .35m^{-1} \text{ generations.}$$

Discussion

This study has confirmed that significant variation in gene frequencies among local populations of sexually reproducing pond invertebrates is a general phenomenon. The extent of the divergence noted in this study is lower than values reported in prior studies of temperate zone cladocerans that, because of their parthenogenetic reproduction, are able to found populations from single individuals. However, the genetic divergence is considerable in light of the compactness of the habitats studied. Values of F_{ST} were consistently about 0.1 among populations only tens of meters apart. There was an obvious difference in the pattern of variation between sites, with

Figure 3-1

Decay of gene frequency differences (F_{ST}) over 2000 generations. These simulations examine the effect of variation in population sizes ($N=10^4$, 10^5, 10^6) for each of three dispersal amounts ($\underline{Nm}_e=1$, 5, 10; $\underline{mN}_e=K$).

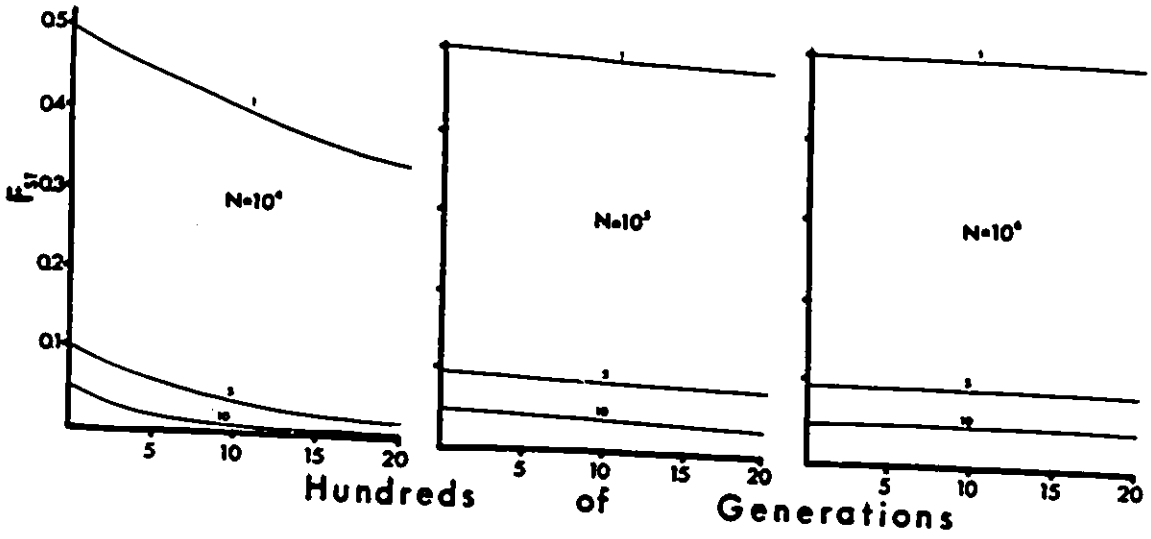


Table 3-3

Estimates of the half-life of decay of genetic differentiation among populations at various levels of gene exchange and population sizes. Estimates were calculated using data from the computer simulated decay curves. The values in parentheses are those expected from our relation for $\underline{H}$ (see text p. 67).

	$\underline{N}=10^4$	$\underline{N}=10^5$	$\underline{N}=10^6$
$\underline{mN}_e=1$	3467.5 (3500)	34,653.8 (35,000)	346,504.2 (350,000)
$\underline{mN}_e=5$	693.5 (700)	6931.1 (7000)	69,242.0 (70,000)
$\underline{mN}_e=10$	349.3 (350)	3467.5 (3500)	34,615.0 (35,000)

genetic differentiation lower at Igloolik than at Churchill.

The estimates of dispersal from gene differentiation were not correlated with the apparent dispersal capabilities of taxa, either within sites or overall. This result suggests that there may be no simple relationship between dispersal and the level of gene frequency divergence. While it is possible that dispersal agents operate more effectively on some dispersal enhancement characters than others, there was also no obvious linkage between local dispersal as inferred from gene frequency data and species distributions. For example, both Branchinecta paludosa and Simocephalus vetulus have a broad distribution over the Canadian arctic, yet their Churchill populations showed the largest gene frequency differences suggesting low dispersal ability.

Each site represents an approximation of the situation considered by Slatkin's (1977) Model II, recently extended by Wade and McCauley (1988), where populations exchange dispersers and become established from within a collection of populations. Equilibrium gene frequency differentiation values as high as those we observed can be accounted for using Wade and McCauley's (1988) model. However, population extinction rates and intrinsic differences between the number of colonists and dispersers are required in their model. Although I doubt the latter condition is met, there are no data to rule it out. On the other hand, the population extinction probabilities required by their model (up to 0.05) to account for the level of divergence observed

are unlikely. Most of the taxa studied produce resting eggs that can remain dormant in pond sediments for several years, hence, several generations of total prereproductive mortality are required for extinction of populations. Indeed, deliberate prereproductive extermination experiments conducted over a 3 year period (P.D.N. Hebert, unpublished data) failed to extirpate local copepod populations. Moreover, surveys of species composition in the Churchill area over a 10 year period have indicated that species assemblages in single ponds are stable. Both lines of evidence suggest that extinction rates are low.

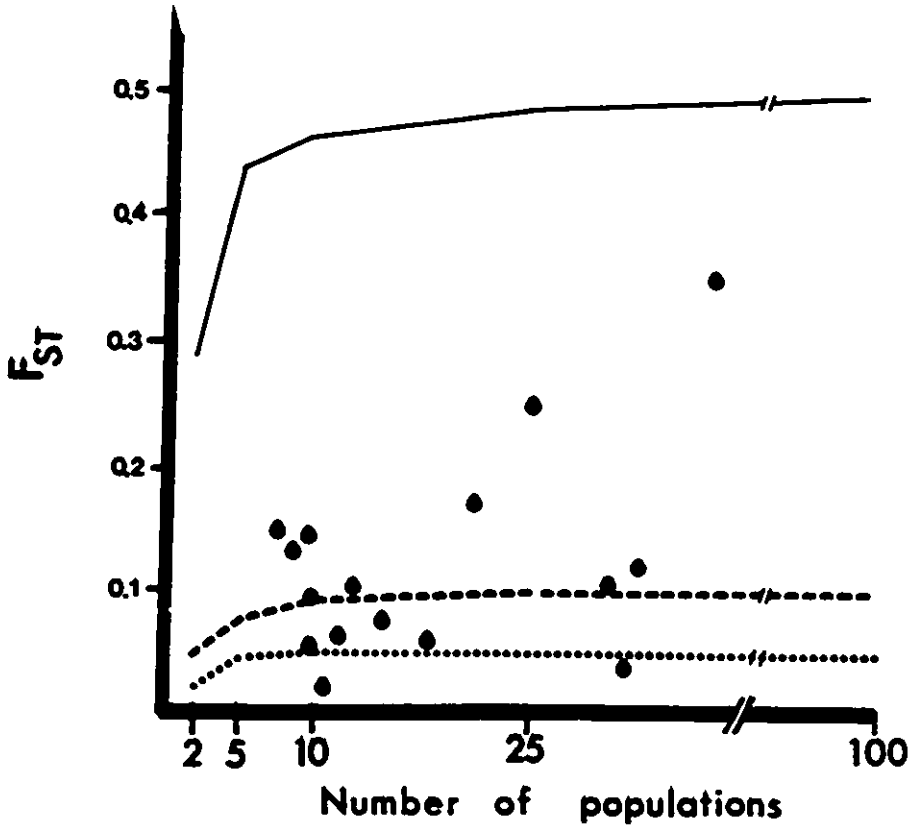
The computer simulation has provided an alternate nonequilibrium interpretation of the gene frequency divergences. The simulation confirmed that the gene frequency differentiation among passively dispersed taxa is expected to be initially high, if populations are founded from fewer than 10 individuals. The simulation also showed that when populations founded from few individuals undergo rapid growth before exchanging a modest number of migrants, gene frequency differences that arise during founding can persist for hundreds or even thousands of generations. The persistence of these differences depends upon the percentage of individual populations that disperses each generation. When population sizes are large, migration rates must be extremely high to rapidly erode the gene frequency differences that arise during founding. When dispersal rates are more modest, gene frequency differentiation can persist for an extended period. For example, an array of

populations averaging 10^6 individuals would require 3500 years to decay an initial F_{ST} of 0.3 to 0.15 if each population received 100 migrants per generation.

The population sizes employed in these simulations are modest for many invertebrate taxa. Populations greater than 10^6 individuals are common in populations of many freshwater invertebrate taxa (Boileau and Hebert, 1988). The Churchill and Igloolik habitats have a maximum age of 3,000 years, and many are likely less than 2,000 years old. As most of the organisms that inhabit these habitats are univoltine, there have been at most, only a few thousand generations since their establishment. Yet these simulation studies show that with such population sizes it is unlikely that the gene frequency differences among populations represent an equilibrium level of gene flow unless migration rates are enormous. Alternatively, the consistently high level of genetic differentiation among these invertebrates may represent differences established during founding which have not yet eroded. If this explanation is accepted, one can estimate the number of original colonists. Thus, the observed F_{ST} values, when compared to those generated by sampling theory and corrected for the finite numbers of populations sampled (Figure 3-2), suggest that populations of many species have been established by fewer than 5 individuals. Annual dispersal on the same order of magnitude will require several thousand more generations for gene frequencies in these populations to reach their equilibrium values.

Figure 3-2

Shifts in the expected mean of F_{ST} as a result of variation in the number of founders per population (----- 1; - - - 5; ·····10). and the number of populations sampled. Data points are the observed F_{ST} and number of populations sampled for 15 taxa studied at two sites in northern Canada.



Wright's model and Slatkin's, (1981, 1985) extensions have been employed by an increasing number of workers to estimate gene flow among populations. Some have found that gene frequency differences correlated poorly with other dispersal capability criteria (Varvio-Aho, 1983; Singh and Rhomberg, 1987; Liebherr, 1988). The present analysis on pond populations has demonstrated that equilibrium models must be employed with caution to estimate gene flow because the prerequisite condition seems unlikely to be met in many cases. Many invertebrate taxa exist in large populations that might require thousands of years to erode gene frequency differences that arise during colonization. The frequent recolonization of habitats also acts to counter the erosion of gene frequency divergence (Wade and McCauley, 1988). Gene frequency distribution, therefore, reflects a dynamic interaction of founder number with migration rate, population sizes and age. Detailed information on these parameters is necessary in order to interpret data collected on the genetic structure of populations. However, when such information is gathered the interpretation is no longer limited to the estimation of dispersal rates, but in some cases may also be used to estimate the number of colonists that established the populations.

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Chapter IV

Genetic consequences of Postglacial Colonization by Pond Dwelling Copepods

Introduction

Freshwater habitats in most of Canada have been available to colonists for only 5000 to 20,000 years. Many of the zooplankton taxa that have colonized these habitats rely on passive dispersal and seem to have encountered difficulty in colonizing the vast areas vacated by the continental ice masses (Segerstrale, 1957; Ricker, 1959; Johnson, 1964; Holmquist, 1966; Bowman and Long, 1968; Dadswell, 1974; Carter et al., 1980; Hebert and Hann, 1986, Black et al., 1986). Thus, biogeographic studies on zooplanktonic crustaceans have revealed that some species are absent in regions that seem similar physically and climatically to those that are occupied (Dadswell, 1974; Hebert and Hann, 1986). For example, a deepwater lacustrian fauna is restricted, in eastern North America, to basins which were formerly occupied by glacial lakes and brackish seas (Dadswell, 1974). Similarly, the copepods have been cited as slow dispersers, because few taxa are present in regions distant from those that were unglaciated (Hebert and Hann, 1986).

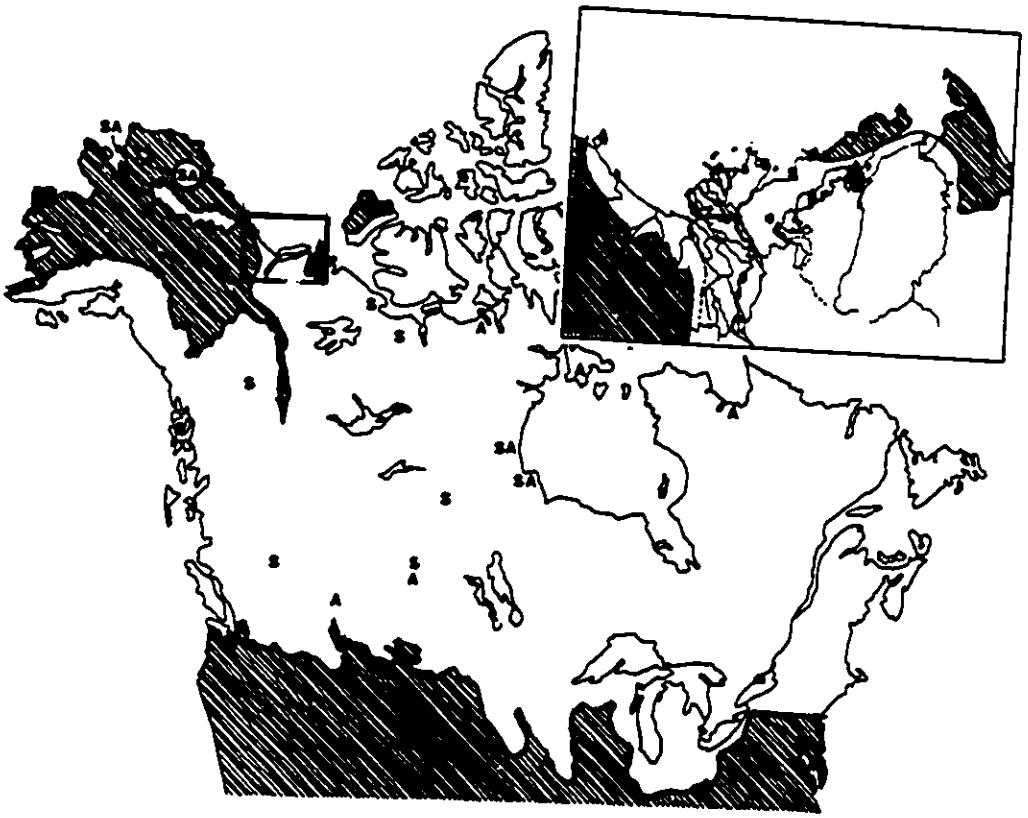
In theory, organisms that colonize new habitats lose genetic variability when their populations are founded by a few individuals (Mayr, 1963; Nei et al., 1975). However, genetic studies of natural populations have shown only minor

changes in variability due to colonization (Schwaegerle and Schaal, 1979; Berlocher, 1984; Parkin and Cole, 1985; Baker and Moeed, 1987; St. Louis and Barlow, 1988) and during dispersal (Bryant et al., 1981; Easteal, 1985). These empirical studies have concentrated on actively dispersing, terrestrial taxa while other organisms such as aquatic taxa, which might be especially affected due to their reliance on passive dispersal, have not been studied over broad areas. Zooplankton species that have become widespread by passive dispersal into discrete pond habitats should be particularly susceptible to the attenuation of genetic variation as a result of multiple founder events.

The zooplankton fauna of northern Canada is particularly suitable for such studies because much of the area was glaciated until 18-20 thousand years ago during the late Wisconsin sub-stage of the Pleistocene. Following deglaciation, organisms recolonized the previously glaciated areas from stocks that persisted during the Pleistocene in unglaciated refugia (Prest, 1976; 1984; Figure 4-1) and presently, many species show broad distributions in arctic Canada. For example, two copepod species, Heterocope septentrionalis and Hesperodiaptomus arcticus, are reported in arctic and alpine habitats from much of the area west of Hudson Bay (Figure 4-1). Heterocope septentrionalis occurs most commonly in tundra pond habitats from Alaska and Yukon to western Hudson Bay (Marsh, 1920; Hooper, 1947; Reed, 1962; Tash and Armitage, 1967; Tash, 1971; Reed, 1963; Hebert and Hann, 1986). It has also been reported from

Figure 4-1

Maximum Wisconsin glaciation and the distributions of Heterocope septentrionalis (S) and Hesperodiaptomus arcticus (A) in North America. Maximum ice boundaries according to Prest, (1976, 1984) and Rampton (1982, 1988). Hatched areas represent unglaciated regions. Dashed line in inset represents Late Wisconsin maximum (~12,000 years BP).



interior British Columbia (Carl, 1940), Saskatchewan (Wilson, 1958) and possibly Massachusetts (Pearse, 1906). Hesperodiaptomus arcticus is similarly distributed in tundra pools from Alaska (Marsh, 1920; Reed, 1962; Tash and Armitage, 1967; Tash, 1971) to Hudson Bay (Reed, 1963; Hebert and Hann, 1986). However, it is also common in alpine ponds in the Canadian Rockies (Anderson, 1968, 1971; 1974) and in prairie sloughs (Wilson, 1953; Hammer and Sawchyn, 1968) as well as east of Hudson Bay near Ungava (Reed, 1963; J. C. H. Carter, personal communication).

The present copepod populations represent descendants of ancestors that were confined to either the Beringian refuge and/or to refuges south of the ice sheet. Populations in proximity to a refuge would be expected to possess the highest genetic diversity, while populations which were derived from them would possess only the diversity of the original colonists and any new mutations that had accumulated in their descendants. The purpose of this study was to examine patterns of genetic relatedness and diversity in these two widespread copepod species to provide information on their postglacial dispersal routes across arctic Canada. Populations of Heterocope septentrionalis and Hesperodiaptomus arcticus were sampled from sites across their distributions and an effort was made to examine populations in the vicinity of potential refugia in unglaciated regions of the western cordillera and Alaska.

Material and Methods

Collections

Samples were collected by net from 15 sites (Figure 4-2; Appendix I) between 1984 and 1988. Both species were present at five arctic sites; three in the western arctic (Mackenzie Delta, Tuktoyaktuk Peninsula, Paulatuk) and two in the eastern arctic (Churchill, Eskimo Point). Animals were removed from ponds and held alive briefly (2-3 days) until electrophoresis was completed.

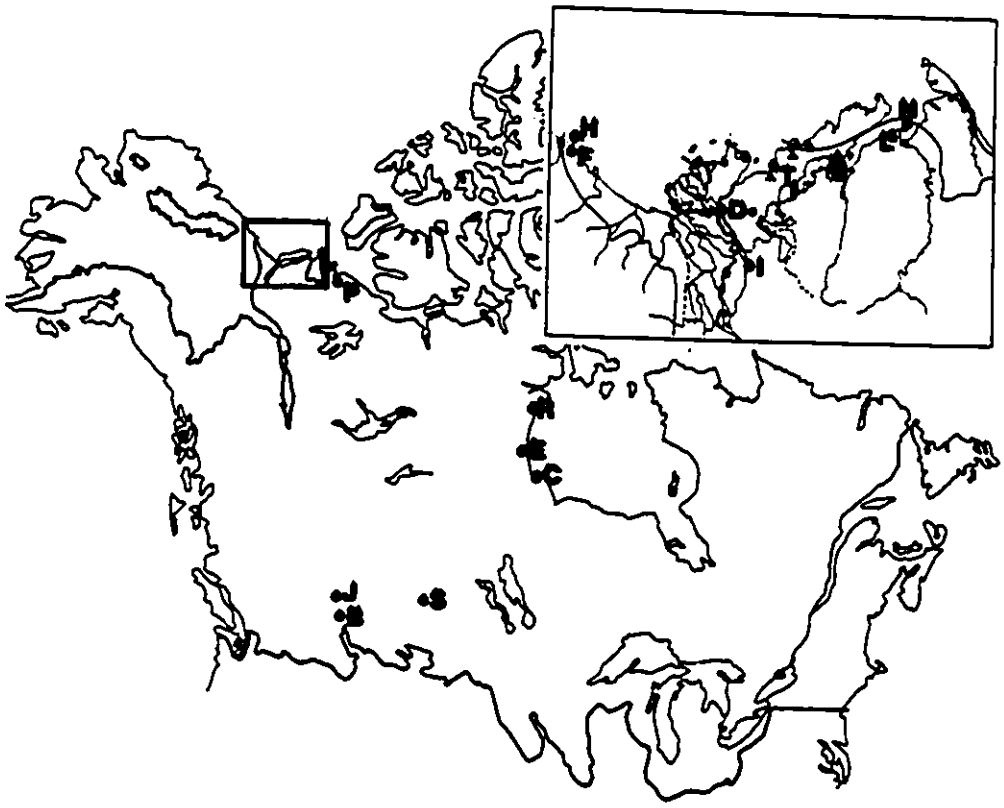
Electrophoresis

Normally 24-48 animals from a population were individually homogenized, electrophoresed and stained for 15 enzymes including: aldehyde oxidase (AO), amylase (AMY), arginine phosphokinase (APK), fumarase (FUM), glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), mannose phosphate isomerase (MPI), leucylglycine and phenylalanylproline peptidases (PEP-C and PEP-D), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and xanthine dehydrogenase (XDH). Electrophoresis buffers and stain recipes were all according to Hebert and Beaton (1989).

An effort was made, within each species, to analyse the entire array of enzymes in all populations in order to detect rare variants, but in some cases this was not possible due to insufficient animals and/or failures of stains. One to several populations or sites were run on a

Figure 4-2

Collection sites for Heterocope septentrionalis and Hesperodiaptomus arcticus in relation to approximate continental ice boundaries. Ice boundaries as in Figure 4-1. Sites: H-Herschel Island; F-Firth River; D-Delta; I-Inuvik; T-Tuktoyaktuk, includes 3 triangles on Tuk peninsula; P-Paulatuk; R-Rankin Inlet; E-Eskimo Point; C-Churchill; J-Jasper; B-Banff; S-Saskatoon.



gel together to compare the mobilities of electromorphic patterns. All allozymes in populations from the Hudson Bay coastline were compared to allozymes of known mobility from Churchill conspecific populations. Allozymes of known mobility from Churchill formed the basis of comparisons to other populations from prairie and western Canada.

Analysis

In order to confirm that the electrophoretic patterns were the products of single enzyme loci and that the populations studied were randomly mating, X^2 tests were conducted on all polymorphic enzyme systems for which the sample size was greater than 25. Phenotype classes with expected values less than 1 were pooled with another class. Bands visualized on gels were considered the products of single enzyme loci if they were monomorphic, or if the variant patterns were consistent with the known quaternary structure of the enzyme (Chapter 2) and they conformed to Hardy-Weinberg expectations (HWE) within populations. Nomenclature for loci followed Boileau and Hebert (1988a). Alleles were numbered according to their anodal mobility, the fastest being #1 and next fastest #2 and so on. Allozymes of similar mobility in different populations were considered the products of the same allele, unless it could be confirmed by juxtaposition on a gel, that they differed. This is a conservative criterion when estimating genetic distances between populations.

In order to determine the genetic relatedness between

all population pairs for each species, a matrix of genetic identities (Nei, 1978) was calculated and analysed using the unweighted pair-group (UPGMA) clustering algorithm of BIOSYS1 (Swofford and Selander, 1981) to produce a dendrogram. Only 22 populations of H. septentrionalis and 27 populations of H. arcticus, for which all polymorphic enzymes had been examined, were included in the cluster analysis.

Four other estimates of genetic diversity were calculated from the gene frequencies of all the populations studied within sites. The percentage of polymorphic loci, using two criteria (P_{99} and P_{95}, frequency of most common allele $\leq$.99 and $\leq$.95 respectively) and number of alleles/locus (A) were calculated for single populations and also after pooling populations from each site because previous analyses (Boileau and Hebert, 1988b; Chapter 2) had revealed that local populations at a site usually contain the same allelic array. Estimates of the mean heterozygosity within populations (H_s) for each locus were averaged over all loci. These estimates were averaged among populations (unweighted) for the site. This method avoids inflating the estimates due to the Wahlund effect which would be greatest where gene frequency divergence was greatest.

Unbiased heterozygosity ($H_e=2n(1-\bar{\sum} p_i^2)/(2n-1)$;Nei, 1978) was used as a measure of diversity within individual populations because the components of variance due to loci and populations could be separated. Loci that were

polymorphic within taxa over the entire distribution and populations that had been examined for the complete array of polymorphic enzyme loci were included in this analysis. Differences in the variability among sites were examined by ANOVA of angularly transformed heterozygosities with sites nested within four regions: western arctic, western cordillera, prairie and Hudson Bay.

Results

Copepods were collected from 58 populations at 15 sites (1-6 populations/site) throughout North America. Heterocope septentrionalis was collected from 31 populations at 10 sites in arctic tundra habitats only. Although the species has been reported in prairie and alpine sites, none were obtained from these locations despite intensive sampling. Hesperodiaptomus arcticus was obtained from 27 populations at 10 sites in arctic, alpine and prairie habitats and all were examined for all enzyme loci.

Allozyme Variation Within Taxa

The 15 enzyme systems studied were coded by 22 loci in Heterocope septentrionalis , 10 of which were monomorphic in all populations at all sites (APK-3, APK-4, AO-1, AO-2, IDH-2, LDH, MDH-2, ME, PEP-C and PEP-D). Polymorphisms at the remaining loci (no minimum frequency criterion) in the 31 populations studied were coded by 2 to 5 alleles (Table 4-1). Hesperodiaptomus arcticus was considerably more variable, with only 3 of 20 loci (AO, IDH-1 and LDH)

Table 4-1

Heterocope septentrionalis allele frequencies for 12 polymorphic enzyme loci surveyed in 31 populations from 10 sites in arctic and subarctic Canada. (*=deviations from HWE significant, P>.05)

Locus/		Firth R.		Herschel		Delta		Tuktoyaktuk			
Allele		1-5	1-12	3-3	3-6	5-2	5-4	T4	P2	6-2	7-1
	n	26	51	55	27	51	27	50	44*	44	40
AMY-3	1	1.0	.98	1.0	1.0	1.0	1.0	.84	.34	.78	.95
	2		.02					.16	.66	.22	.05
	n	12	27	52*	26	27	27	21	44*	14	35
AO-3	1	1.0	.94	.52	.71	1.0	.96	.26	.47	.86	.51
	2		.06	.48	.29		.04	.74	.53	.14	.49
	n	26	27	31	27	27	27	47	44	31	31
APK-1	1	1.0	.96	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2		.04								
	n	26	27	31	27	27	27	47	44	31	31
APK-2	1		.02								
	2	1.0	.98	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	3										
	n	26	51	55	27	51	27	47	44	20	16
FUM	1					.12	.37	.17	1.0	1.0	.97
	2	1.0	1.0	1.0	1.0	.88	.63	.83			.03
	n	14	27	27	27	27	27	35	20	44	40
GOT-2	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.85	1.0	1.0
	2								.15		
	n		48*	48		51	27	20	24	20	40
IDH-1	1	---	.42	.47	---	1.0	1.0	1.0	1.0	.72	1.0
	2		.58	.53						.28	
	n	26	51	55	27	51	27	44	44	44	40*
MDH-1	1		.14	.02	.02	.22	.32	.82	.38	.53	.24
	2	1.0	.86	.98	.98	.78	.68	.18	.61	.43	.75
	3								.01	.04	.01
	n	26	51	55	27	51*	27	47	44	44	40
MPI	1									.16	
	2	.12	.15	.26	.50	.62	.46	.38	.27	.18	.26
	3	.88	.77	.30	.30	.38	.54	.62	.73	.62	.74
	4		.08	.44	.20					.03	

Table 4-1 continued

Locus/ Allele		Firth R. 1-5	Firth R. 1-12	Herschel 3-3	Herschel 3-6	Delta 5-2	Delta 5-4	Tuktoyaktuk T4	Tuktoyaktuk P2	Tuktoyaktuk 6-2	Tuktoyaktuk 7-1
PGI	n	26	51	55	27	50	27	35	44	44	40
	1	.12	.08	.88	.76	.80	1.0	.24		.47	.29
	2	.88	.92	.12	.24	.20		.76	1.0	.53	.71
	3										
PGM	n	26	51	55	27	51	27	45	44	44	40
	1		.02	.12	.41	.17	.06	.19			
	2	.52	.43	.36	.50	.74	.83	.43	.83	.88	.58
	3	.06	.31	.38	.07	.06	.11	.24	.17	.11	.41
	4	.33	.21	.10	.02	.03		.13		.01	.01
	5	.09	.03	.04							
XDH	n	26	27	55	27	51	27	27	24	44	40
	1										
	2	.73	.56	1.0	.98	.52	.74	.91	1.0	1.0	.46
	3	.27	.44		.02	.48	.26	.09			.54

Table 4-1 continued

Locus/ Allele		Inuvik						Liverpool		Paulatuk		
		D2	I18	I20	I1,	I15	I16	10-2	10-4	12-2	12-5	12-7
	n	65	17	54	69	24	20	37	19	44	48	44
AMY	1	.88	.91	.92	.97	1.0	.95	.68	.60	.92	.88	.98
	2	.12	.09	.08	.03		.05	.32	.39	.08	.12	.02
	n	48	17	54				15	16	44	48	41
AO-3	1	.53	.50	.93	---	---	---	.80	.50	.79	1.0	.29
	2	.47	.50	.07				.20	.50	.20		.71
	n	24	27	45	45	24	24	45	14	44	48	21
APK-1	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2											
	n	24	27	45	45	24	24	45	14	44	48	21
APK-2	1											
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	3											
	n	26	10	24				37		39	48	20
FUM	1							1.0	---	1.0	1.0	1.0
	2	1.0	1.0	1.0	---	---	---					
	n	53	17	54			24	32	19	41	24	44
GOT-2	1	.65	1.0	1.0	---	---	1.0	.98	.97	.45	.56	.61
	2	.35						.02	.03	.55	.44	.39
	n	55	27	45*				24	16	24	48	20
IDH-1	1	.41	.67	.77	---	---	---	.62	.50	.40	1.0	1.0
	2	.59	.33	.23				.38	.50	.60		
	n	65	17	54		17		24		39*	69	44
MDH-1	1	.58	.56	.42	---	.56	---	.44	---	.46	.72	.74
	2	.42	.44	.54		.44		.56		.54	.28	.26
	3			.04								
	n	63	27	54	69	27	24	29	19	44	48	44
MPI	1											
	2	.13	.06	.13	.15	.06	.17	.83	.90	.35	.40	.42
	3	.74	.94	.85	.79	.94	.83	.07	.10	.65	.60	.58
	4	.13		.02	.06			.09				

Table 4-1 continued

Locus/ Allele		Inuvik						Liverpool		Paulatuk		
		D2	I18	I20	I1	I15	I16	10-2	10-4	12-2	12-5	12-7
PGI	n	67	27	54	60	24	23	45*	19	44	48	44
	1	.02		.06	.24	.17	.35	.20	.26	.22	.09	.18
	2	.98	1.0	.94	.74	.83	.65	.80	.74	.78	.91	.82
	3				.02							
PGM	n	62	27	54	65	24	22	37	19	44	48	44
	1	.57	.65	.76	.08	.44	.25	.88	.87	.99	.99	.96
	2	.36	.18	.23	.53	.29	.46	.03		.01	.01	.04
	3	.06	.17	.01	.23	.17	.29	.09	.13			
	4	.01			.16	.10						
	5											
XDH	n	51	27	41			24	37	19	44	48	44
	1	.02										
	2	.79	.52	.46	---	---	1.0	.84	.80	.08	.04	.03
	3	.19	.48	.54				.16	.21	.92	.96	.97

Table 4-1 continued

Locus/ Allele		Churchill						Eskimo Point			Rankin Inlet
		TW2	T1	T35	T37	T77	T92	ET6	ET12	ET13	RT8
	n	35	48	60	60	48	48	22	48	48	31
AMY	1	.66	.88	.76	.81	.82	.53	.98	1.0	1.0	.60
	2	.34	.12	.24	.19	.18	.47	.02			.40
	n	36	47	72	48	22	24	24	48	48	24
AO-3	1	1.0	.76	1.0	.71	.54	.90	1.0	1.0	1.0	1.0
	2		.24		.29	.46	.10				
	n	36	48	36	36	72	24	48	48	48	48
APK-1	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2										
	n	36	48	36*	36	72	24	48	48	48	48
APK-2	1										
	2	.94	1.0	.62	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	3	.06		.38							
	n	24	24	12	48	48	24	48	48	24	24
FUM	1										
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	n	24	48	36	60	48	24	46	48	48	24
GOT-2	1	.94	1.0	.97	1.0	1.0	1.0	.58	.57	.65	1.0
	2	.06		.03				.42	.43	.35	
	n			60	48	48	24	46*	48	48	20
IDH-1	1	---	---	.27	.40	.99	.73	.16	.62	.29	.45
	2			.73	.60	.01	.27	.84	.38	.71	.55
	n	24	24	72	48	72	48	48	48	48	24
MDH-1	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.80
	2										.20
	3										
	n	36	38*	72*	72	36	24	62	48	48	
MPI	1	.94	.35	.18	.10	.22		.02	.09	.12	---
	2	.06	.57	.82	.90	.78	1.0	.98	.91	.88	
	3		.08								
	4										

Table 4-1 continued

Locus/ Allele		Churchill						Eskimo Point			Rankin Inlet
		TW2	T1	T35	T37	T77	T92	ET6	ET12	ET13	RT8
PGI	n	36	48	48	48	47	48	63	48	48	56
	1							.01			
	2	.17	.30	.18	.32	.63	.51	.40	.45	.52	.20
	3	.83	.70	.82	.68	.37	.49	.59	.55	.48	.80
PGM	n	36	48	48	48	60	48	24	48	48	46
	1		.31	.54	.48	.29	.29	.25	.07		.14
	2	.93	.41	.44	.50	.41	.59	.21	.23	.19	.21
	3	.07	.25	.02	.01	.30	.12	.46	.70	.80	.42
	4		.03		.01			.02		.01	.17
	5							.06			.05
XDH	n	24	24	36	36	48	48	48	48	48	24
	1										
	2		.38					1.0	1.0	.98	1.0
	3	1.0	.62	1.0	1.0	1.0	1.0			.02	

Table 4-2

Hesperodiaptomus arcticus s. l allele frequencies for 17 polymorphic enzyme loci surveyed in 27 populations from 10 sites throughout arctic, alpine and prairie Canada.

		Delta	Tuktoyaktuk					Nicholson Pt.		Paulatuk	
Locus/ Allele		5-2	TK6	TK11	TK13	TK14	EH5	8-2	11-1	11-2	12-2	12-3
AMY	n	12	24	24	46	48	44	20	16	12	20	44
	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2											
APK-1	n	12	24	44	46	46	44	20	28	24	20	44
	1											
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	3											
	4											
APK-2	n	12	24	44	46	46	44	20	28	24	20	44
	1											
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
APK-4	n	12	24	44	46	46	44	20	28	24	20	44*
	1											
	2								.50	.56	.65	.74
	3	1.0	1.0	1.0	1.0	1.0	1.0				.35	.23
	4							1.0	.50	.44		
FUM	n	12	24	24	46	48	44	20	28	24	20	44
	1										.50	.53
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.50	.47
GOT-1	n	12	38	24	46	48	46	20	28	24	20	44
	1							1.0	.98	1.0	1.0	1.0
	2	.12	.59	.56	.15	.58	.20		.02			
	3	.88	.41	.44	.85	.42	.80					
GOT-2	n	12	38	24	46	48	46	20	28	24	20	44
	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.81	1.0	1.0
	2									.19		
IDH-2	n	12	24	24	46	48	44	20	28	12	20	24
	1											
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
MDH-1	n	12	16	24	46	47	34	40	28	24	20	44
	1							.10	.07	.29		.06
	2		.09		.01	.04	.26	.90	.93	.71	1.0	.94
	3	1.0	.91	1.0	.99	.96	.74					

Table 4-2 continued

Locus/ Allele		Delta	Tuktoyaktuk					Nicholson Pt.		Paulatuk	
		5-2	TK6	TK11	TK13	TK14	EH5	8-2	11-1	11-2	12-2	12-3
MDH-2	n	12	24	24	46	48	36	40	28	24	20	44
	1							1.0	1.0	1.0	1.0	1.0
	2	1.0	1.0	1.0	1.0	1.0	1.0					
ME	n	12	24	24	46	48	36	40	28	24	20	44
	1	1.0	1.0	1.0	1.0	1.0	1.0					
	2							1.0	1.0	1.0	1.0	1.0
MPI	n	12	38	44	46	47	44	40	28	24	20	44
	1											
	2											
	3							.10	.04	.71	1.0	1.0
	4							.90	.96	.29		
	5	.08		.01	.03	.06	.07					
	6	.92	1.0	.99	.97	.94	.93					
PEP-C	n	12	24	24	46	48	36	20	12	24	20	44
	1											
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PEP-D	n	12	24	24	46	48	24	20	28	24	20	44
	1										.48	.39
	2							1.0	.61	.56	.48	.52
	3	1.0	1.0	1.0	1.0	1.0	1.0					
	4										.02	.09
	5								.39	.44	.02	
PGI	n	12	24	24	46	48	44	20	40	24	20	44
	1											
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	3											
PGM	n	12	30	22	46	46	44	24	28	24	20	44*
	1											
	2								.05		.15	.84
	3							1.0	.95	1.0	.85	.16
	4	1.0	.71	.77	.56	.74	.88					
	5		.21	.18	.44	.22	.12					
	6		.08	.05		.04						
XDH	n	12	14	33	48	48	24	40	28*	24	19	43
	1	.83	.32	.58	.63	.38	.50					
	2	.17	.68	.42	.37	.62	.50	.64	.45	.15	.45	.45
	3							.36	.55	.85	.55	.55

Table 4-2 continued

Locus/ Allele		Banff/Jasper BF1	BF2	JC	Saskatoon FMEL	5MEL	EMEL	4MEL	Churchill C90	C107	T92
AMY	n	24	24	24	24	24	20	24	24	24	23
	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2										
APK-1	n	48	48	48	48	46	48	46	48	48	47
	1					.01	.01	.01			
	2	1.0	.99	1.0	.97	.94	.96	.92	1.0	1.0	1.0
	3				.03	.05	.03	.07			
	4		.01								
APK-2	n	48	48	48	48	46	48	46	48	48	47
	1					.01		.01			
	2	1.0	1.0	1.0	1.0	.99	1.0	.99	1.0	1.0	1.0
APK-4	n	48	48	48	48	46	48	46	48	48*	47
	1										
	2	.90	.75	.75	.11	.28	.32	.38	.80	.82	.64
	3	.10	.25	.25	.55	.48	.46	.44	.20	.18	.36
	4				.28	.24	.22	.18			
FUM	n	24	48	24	48	44	28	46	20	62	73
	1		.02		.99	.94	.98	1.0	.35		.57
	2	1.0	.98	1.0	.01	.06	.02		.65	1.0	.43
GOT-1	n	48	48	24	24	43	52	46	32	36	35
	1	1.0	1.0	1.0	.67	.72	.55	.85	.02		1.0
	2				.33	.28	.45	.15	.98	1.0	
	3										
GOT-2	n	48	48	24	24	43	52	46	32	36	35
	1	.10	.22	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	.90	.78								
IDH-2	n	24	24	48	24	24	20	24	24	24	24
	1			.05							
	2	1.0	1.0	.95	1.0	1.0	1.0	1.0	1.0	1.0	1.0
MDH-1	n	24	24	24	48	44	50	46	24	24	36
	1										
	2	1.0	1.0	1.0	1.0	.93	.99	.92	1.0	1.0	1.0
	3					.07	.01	.08			

Table 4-2 continued

Locus/ Allele		Banff/Jasper BF1	BF2	JC	Saskatoon FMEL	5MEL	EMEL	4MEL	Churchill C90	C107	T92
MDH-2	n	24	24	24	48	44	50	46	24	24	36
	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2										
ME	n	24	24	24	48	44	50	46	24	24	36
	1										
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
MPI	n	24	24	48	48	44	27	46	44	47	24
	1				.29	.28	.37	.25	.44	.22	
	2				.60	.51	.54	.47	.44	.78	
	3	1.0	1.0	.73	.11	.21	.09	.28	.12		.44
	4			.27							.56
	5										
	6										
PEP-C	n	24	24	24	48	44	50	46	24	24	12
	1				1.0	1.0	1.0	1.0	1.0	1.0	
	2	1.0	1.0	1.0							1.0
PEP-D	n	24	24	48	24	24	20	24	24	60	24
	1										
	2		1.0	.40	1.0	1.0	1.0	1.0	1.0	1.0	.88
	3										
	4	1.0		.60							.12
	5										
PGI	n	48	48	48	48	43	29	46	24	24	35
	1				.23		.09				
	2	.99	.96	.56	.66	.86	.69	.81	1.0	1.0	1.0
	3	.01	.04	.44	.11	.14	.22	.19			
PGM	n	24	24	24	48	43	38	46	24	24	24
	1				.40	.36	.41	.30			
	2	1.0	1.0	1.0	.43	.45	.42	.46	1.0	1.0	.98
	3				.17	.19	.17	.24			.02
	4										
	5										
	6										
XDH	n	44	44	24	24	24	20	24	36	48	36
	1										
	2	.61	.91	1.0					.04	.97	.74
	3	.39	.09		1.0	1.0	1.0	1.0	.96	.03	.26

Table 4-2 continued

Locus/ Allele		Churchill		Eskimo Point		Rankin Inlet	
		C44	A21	ET12	ET13	RT5	RT8
AMY	n	48	24	20	24	44	42
	1	1.0	1.0	1.0	1.0	.94	.83
	2					.06	.17
APK-1	n	47	48	20	52	44	42
	1						
	2	1.0	1.0	1.0	1.0	.96	1.0
	3						
	4					.04	
APK-2	n	47	48	20	52	44	42
	1						
	2	1.0	1.0	1.0	1.0	1.0	1.0
APK-4	n	47	48	20	52	44	42
	1			.05	.01		
	2	.94	.71	.78	.85	.51	.34
	3	.06	.29	.17	.14	.49	.66
	4						
FUM	n	48	36	20	49	22	22
	1	.09	.12	.88	.83	.96	.76
	2	.91	.88	.12	.17	.04	.24
GOT-1	n	48	24	20	21	24	24
	1	1.0	1.0	1.0	1.0	1.0	1.0
	2						
	3						
GOT-2	n	48	24	20	21	24	24
	1	1.0	1.0	1.0	1.0	1.0	1.0
	2						
IDH-2	n	48	24	20	24	24	24
	1						
	2	1.0	1.0	1.0	1.0	1.0	1.0
MDH-1	n	48	24	20	24	24	24
	1						
	2	1.0	1.0	1.0	1.0	1.0	1.0
	3						

Table 4-2 continued

Locus/ Allele		Churchill		Eskimo Point		Rankin Inlet	
		C44	A21	ET12	ET13	RT5	RT8
MDH-2	n	48	24	20	24	24	24
	1	1.0	1.0	1.0	1.0	1.0	1.0
	2						
ME	n	48	24	20	24	24	24
	1						
	2	1.0	1.0	1.0	1.0	1.0	1.0
MPI	n	48	46	20	49	94	42
	1						
	2						
	3	.27	.15	.42	.54	.48	.83
	4	.73	.85	.53	.36	.51	.17
	5			.05	.10	.01	
	6						
PEP-C	n	48	24	20	24	24	24
	1						
	2	1.0	1.0	1.0	1.0	1.0	1.0
PEP-D	n	48	48	20	52	72	48
	1			.05	.05		
	2	.86	.77	.92	.91	.99	1.0
	3						
	4	.14	.23	.03	.04	.01	
	5						
PGI	n	48	24	20	45	72	48
	1						.06
	2	1.0	1.0	1.0	1.0	.99	.94
	3					.01	
PGM	n	48	24	20	21	60	24
	1						
	2	.99	1.0	1.0	1.0	.99	1.0
	3	.01				.01	
	4						
	5						
	6						
XDH	n	48	24	20	24	44	22
	1						
	2	.04	.33			.75	1.0
	3	.96	.67	1.0	1.0	.25	

invariant. Variation at polymorphic loci was coded by 2 to 6 alleles (Table 4-2).

Allozyme Variation within Populations

Tests for deviations from HWE in Heterocope populations revealed that 13 of 158 tests (8.2%), were significant (Table 4-1; P X^2<.05) with most (10) resulting from slight heterozygote deficiencies. Significant deviations were observed in only 7 (5.9%) of 117 comparisons in Hesperodiaptomus (Table 4-2). However, two populations at Churchill (C13, C59; Table 4-3) showed consistent deviations from HWE at 3 loci (MPI, GOT-1 and PEP-C) resulting from the absence of heterozygote classes.

Heterozygotes were completly absent in these Hesperodiaptomus populations at the GOT-1 and PEP-C loci despite the presence of two alleles in both. There was also a clear link between the variants at these two loci. Individuals with the GOT-1^1 invariably possessed the PEP-C^2 allele and those with the GOT-1^2 allele had the PEP-C^1 allele (Table 4-3). If random mating was occurring between individuals with these alleles it is unlikely ($P=3.72\times10^{-12}$) that only the two homozygote classes could be observed among 98 individuals. Individuals with MPI^1 and MPI^2 alleles were also homozygous for GOT-1^1 and PEP-C^2 while those with MPI^3, MPI^4 and MPI^5 alleles were homozygous for the alternate GOT-1 and PEP-C alleles. Of the five populations assayed at Churchill, two (C90 and C107) corresponded to the animals with the GOT-1^{22}/PEP-C^{11}/MPI^{faster} genotypes. Three

Table 4-3

Three locus genotypes of 118 Hesperodiaptomus arcticus s. l. assayed from Pond C13 and Pond C59, Churchill.

# of animals	Genotype MPI	GOT-1	PEP-C
	Pond C13 (July 24, 1987)		
6	11	22	11
3	12	22	11
9	22	22	11
15	33	11	22
33	34	11	22
31	44	11	22
1	35	11	22
	Pond C59 (July 26, 1987)		
8	12	22	11
2	22	22	11
1	33	11	22
3	34	11	22
5	44	11	22
1	24*	11	22

* a single animal observed that could represent introgression of MPI^{faster} system into MPI^{slower} system.

populations (T92, C44 and A21) corresponded to the GOT-1^{11}/MPI^{22}/MPI^{slower} genotypes.

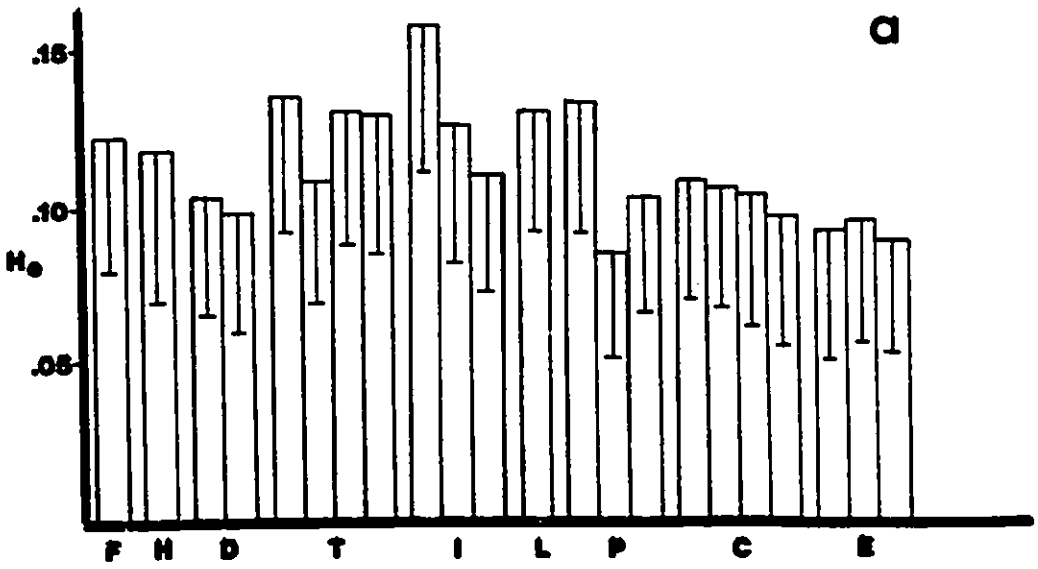
Geographic Shifts in Genetic Variability and Allelic Arrays

Estimates of individual heterozygosities (unbiased H_e) within populations fluctuated among sites in both Heterocope septentrionalis and Hesperodiaptomus arcticus (Figure 4-3). The means of all measures of variability were lower in Heterocope septentrionalis at eastern sites than western sites (Table 4-4). For example, western sites of Heterocope possessed higher mean heterozygosities (H_e=.116) than eastern sites (H_e=.103) and the differences between western and eastern populations that were analysed for the entire array of loci (Figure 4-3a) were significant (ANOVA; $F_s=11.8<F_{.005[1,20]}$). In H. arcticus no geographic pattern in variability was seen, although differences among populations were more pronounced than those of H. septentrionalis (Figure 4-3b).

Heterocope septentrionalis gene frequencies within sites were generally similar and adjacent sites usually had a similar array of alleles at most polymorphic loci (Table 4-1). Populations within a site were never monomorphic for alternate alleles, although two loci (FUM and XDH) showed substantial shifts in frequency between the Delta and Paulatuk sites. Thus the average frequency of the FUM^2 allele was 0.75 at the Delta and Tuktoyaktuk sites but the allele was absent at Paulatuk. Similarly, XDH^2 was nearly absent from Paulatuk, but was the most frequent allele west

Figure 4-3

Geographic shifts in heterozygosity among populations of Heterocope septentrionalis (a) and Hesperodiaptomus arcticus s. l (b). Populations are arranged by sites approximately west (left) to east (right). Bars represent heterozygosity, all loci considered, with standard errors.



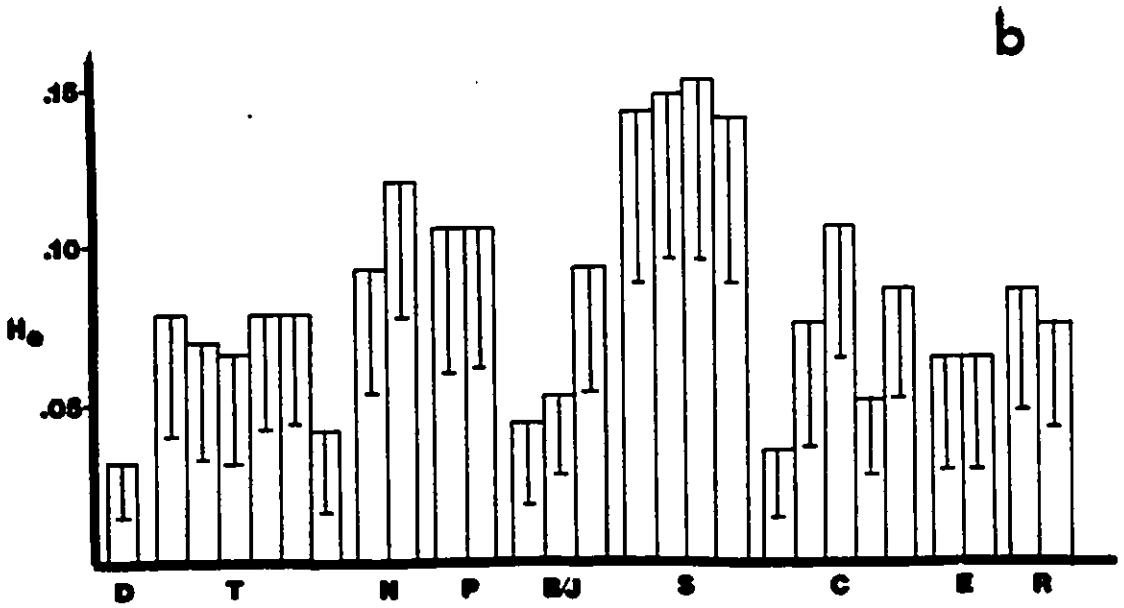


Table 4-4

Genetic variability measures for 22 loci in Heterocope septentrionalis from 10 sites throughout arctic and subarctic Canada.

Location	n	s	L	P_{99}	P_{95}	A	H_s
Churchill	230	6	22	40.9	36.4	1.54	.104
Eskimo Point	127	3	22	27.3	22.7	1.09	.094
Rankin Inlet	30	1	21*	23.8	23.8	1.38	.111
Eastern Site Means				30.7	27.6	1.34	.103
Firth River	58	2	22	40.9	27.3	1.64	.104
Herschel Island	69	2	22	31.8	22.7	1.41	.116
Mackenzie Delta	64	2	22	31.8	27.3	1.32	.100
Tuktoyaktuk	117	4	22	45.4	45.4	1.68	.126
Inuvik	136	6	22	40.9	40.9	1.59	.139
Paulatuk	116	3	22	40.9	31.8	1.41	.097
Liverpool Bay	47	2	22	40.9	36.4	1.50	.134
Western Site Means				38.9	33.1	1.51	.116

n = average number of individuals analysed per locus
s = total # of populations at site
L = # of loci studied
P_{99} P_{95} = proportion of loci polymorphic, 99% and 95% criteria respectively
A = average # of alleles per locus
H_s = average expected heterozygosity within populations
*Rankin Inlet not included in ANOVA of heterozygosity

of this site. Five alleles whose frequencies ranged from 0.011 to 0.445 in 1-9 western arctic populations (APK-1^2, APK-2^1, MDH-1^3, MPI^4 and XDH^1; Table 4-2)were not seen, however, at the eastern sites. A single rare allele (0.072) at APK-2^3 was observed in two populations at one eastern site (Churchill) but not in the west.

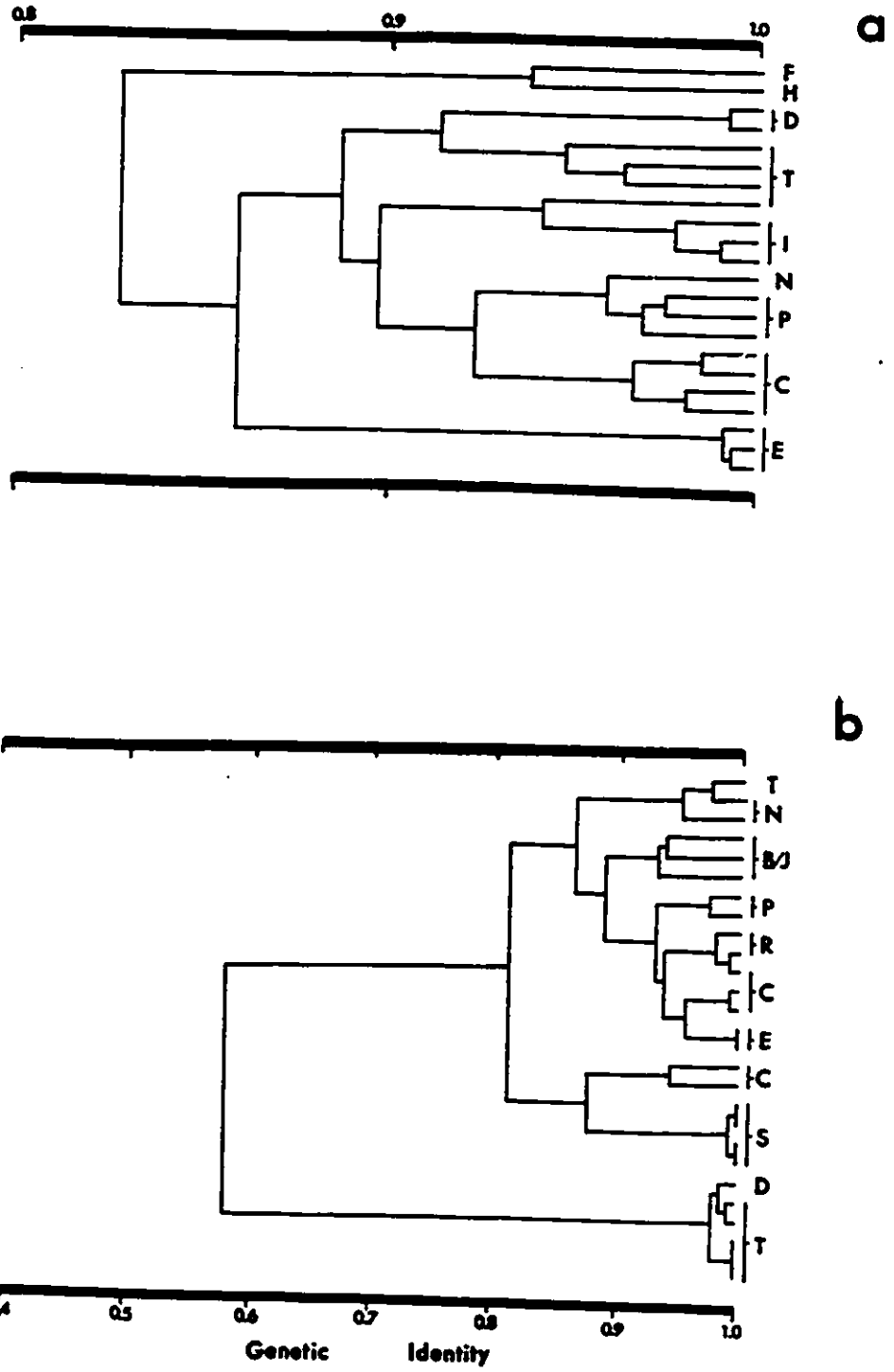
In contrast to the pattern observed in Heterocope, Hesperodiaptomus arcticus allele frequencies varied substantially between sites, and even within some sites. Eight loci (GOT-1, MDH-2, ME, MPI, PEP-C, PEP-D, PGM and XDH) were frequently coded by different alleles in adjacent sites (Table 4-2). For example, Tuktoyaktuk local populations were monomorphic for PEP-D^3 and MDH-2^2 while these alleles were completely absent in the Peninsula population (8-2), only 47 km away. The PEP-D and MDH-2 alleles as well as others at GOT-1, ME, MPI, PGM, and XDH observed at Tuk local populations were only seen at one other site, west of Tuktoyaktuk (Delta) while all sites east of these two localities possessed a different array of alleles.

Genetic Distance Analysis

The average genetic identities (I) of all pairwise comparisons of populations (Appendix V) within the two taxa reflected the differences in allelic arrays. The mean genetic identity of Heterocope populations was significantly higher (0.883 $\pm$.003 S.E., 231 comparisons) than Hesperodiaptomus (0.770$\pm$.008, 351 comparisons ANOVA;

Figure 4-4

Cluster analysis (UPGMA) of Nei's genetic identity in (a) 22 populations of Heterocope septentrionalis and (b) 27 populations of Hesperodiaptomus arcticus. Note: scale difference between a & b.



F=82.23, P<<.001).

Cluster analyses (Figure 4-4) of populations which were analysed for the complete array of loci in both taxa revealed substantial differences in the genetic cohesiveness of these taxa. No branches were less than I=0.82 (Figure 4-4a) in Heterocope and all within site branches were greater than 0.96. By contrast, Hesperodiaptomus populations were separated into three distinct branches with I less than 0.82 (Figure 4-4b). One genetically distinct branch included all of the populations from Tuktoyaktuk and the sole population from Delta. A second major branch linked two of the five Churchill populations with the four Saskatoon populations. The final cluster included eastern populations along Hudson Bay to Tuktoyaktuk in the western arctic as well as the Rocky Mountain populations. The clusters of populations within each of these three branches showed the same level of genetic similarity as that noted in Heterocope.

Discussion

In the past decade, biochemical methods such as enzyme electrophoresis have been recognized as powerful tools for the delimitation of species boundaries (Avise, 1974; Ayala, 1983; Thorpe, 1983). Where reproductively isolated taxa co-occur their detection is simplest, signified by the absence of heterozygous individuals at polymorphic loci. However, if groups of allopatric populations are genetically cohesive within a region and substantially different from populations in another region, it is appropriate to consider them as

different species.

Macrogeographic patterns of genetic variation in the two copepod taxa included in this study were strikingly different. Heterocope septentrionalis showed relatively minor shifts in gene frequencies over its distribution and only subtle geographic differences in the level of genetic variation. Hesperodiaptomus arcticus, by contrast, was separated into three genetically distinct groups of populations. At the Churchill site two of these groups co-ocurred without interbreeding, while the third taxon showed considerable genetic divergence from the remaining members of the group. These observations suggest that Hesperodiaptomus arcticus is not a single taxon but is composed of three reproductively isolated species. These species must be discriminated first, to allow ancient genetic divergences to be separated from the more recent response to postglacial dispersal.

Genetic Divergence of Hesperodiaptomus arcticus s. l.

Allozyme data indicated the presence of two reproductively isolated species within Hesperodiaptomus arcticus sensu lato at Churchill. Specifically, the absence of heterozygote classes and the presence of linkage associations make it clear that two taxa are present. Although mating trials alone can confirm the absence of interbreeding, the allozyme results indicate that interbreeding in nature is either rare or that hybrid offspring are inviable. A single individual (Table 4-3) of 240 examined possessed an allele that could have been the

result of introgression, but mating trials between Isolate C females and Isolate N males were all unsuccessful (personal observation). The reciprocal mating trials could not be conducted because mature females of Isolate N became available in August when Isolate C males were absent.

The genetic distance analysis revealed a third genetically distinct group of H. arcticus populations at Tuktoyaktuk and Mackenzie Delta (Isolate A). These populations were more distinct than the reproductively isolated taxa at Churchill, but did not co-occur with them. However, the presence of diagnostic alleles at seven loci within this taxon and the resultant low genetic identity with both of the other two isolates supports the conclusion that this taxon is reproductively isolated from the other species pair. The average genetic identities between populations are all higher within isolates (Isolate A, $I=0.988\pm.002$; Isolate N, $I=0.908\pm.005$; Isolate C, $I=0.931\pm.017$) than the overall identity of populations confirming that these isolates are genetically cohesive between regions.

Approximate times since divergence (t) can be estimated from the genetic identities of the isolates in order to determine whether the genetic differences can be considered postglacial. Employing Nei's (1987) conservative formula ($t=5\times10^6D$, where $D=-\ln I$), I used D=0.21 for the branch point between Isolates C and N to estimate $t=1.05 \times 10^6$ years. I used D=0.55 for the branch point between Isolate

A and the others to estimate $t=2.75 \times 10^6$ years. These results suggest that the three taxa shared a common ancestor in the Pliocene and that Isolate A diverged from the other two taxa early in the Pleistocene. A further speciation event occurred during the middle Pleistocene, perhaps in response to isolation in different refugia. The results make it clear that it is necessary to consider each isolate separately in relation to the impact of recent glaciation.

Macrogeographic Patterns of Genetic Variation and Postglacial History

In order to consider whether genetic variation has been lost within taxa due to repeated founder effects, as they radiated from glacial refugia, it is appropriate to ignore monomorphic loci because genetic variation cannot be lost at them. The present study revealed that significantly higher heterozygosities were present in Heterocope populations from western than eastern sites. Western populations also possessed several alleles, some in high frequency, that eastern populations lacked while only a single rare allele was restricted to the east. These genetic data suggest that Heterocope septentrionalis did not survive in southern refugia, but rather colonized North America from an Alaskan glacial refugium.

This species was not detected in prairie or Rocky Mountain sites despite intensive sampling effort. Thus, its present distribution also suggests that it was not present in refugia south of the continental ice mass.

Hesperodiaptomus arcticus Isolate A was obtained in

this study at only two sites in the western arctic. If its ancestors were isolated in Beringia and these populations possessed all of the representative variation then this taxon is genetically depauperate. Calanoid copepod heterozygosities average .093 (Chapter 2), but the average heterozygosity within populations of this isolate was only 0.054 (Table 4-5). However, there is a clear need to survey levels of variability in additional populations from the Yukon and Alaska.

Isolate C was more variable in Saskatoon (H_s=0.145) than in Churchill (H_s=0.055) and this difference was significant (F_s=10.29>$F_{.025[1,5]}$). This result suggests that this taxon was confined to regions south of the continental ice mass from which it populated the prairies and Churchill areas.

The western arctic populations of Isolate N apparently have higher diversity (P_{99}, P_{95}, A and H_s, Table 4-5) than those in the cordillera or the east, but the differences in heterozygosity were not significant (F_s=2.02<$F_{.10[2,12]}$).

In summary, prior studies on Hesperodiaptomus arcticus implied that it was a widespread and ecologically tolerent species in North America. However, using genetic methods, this study has identified three reproductively isolated taxa whose geographic distributions are all less widespread. Dispersal efficiencies and ecological characteristics implied by biogeographical studies need to be viewed cautiously in copepods because there may be many more cases

Table 4-5

Genetic variability measures for 20 loci in Hesperodiaptomus arcticus reproductive isolates from 10 sites throughout arctic and subarctic Canada.

Location	n	s	L	P_{99}	P_{95}	A	H_e
		Isolate A					
Mackenzie Delta	12	1	20	15.0	15.0	1.15	.034
Tuktoyaktuk	204	5	20	19.0	23.0	1.26	.074
Means				17.0	19.0	1.20	.054
		Isolate C					
Saskatoon	188	4	20	31.2	40.0	1.61	.145
Churchill	96	2	20	17.5	20.0	1.22	.055
Means				24.4	30.0	1.42	.100
		Isolate N					
Tuk Peninsula	20	1	20	15.0	15.0	1.15	.042
Nicholson Point	52	2	20	27.5	32.5	1.32	.107
Paulatuk	64	2	20	27.5	27.5	1.35	.116
Banff/Jasper	144	3	20	21.7	25.0	1.22	.063
Churchill	141	3	20	23.3	28.3	1.28	.081
Eskimo Point	70	2	20	20.0	20.0	1.35	.065
Rankin Inlet	86	2	20	42.5	30.0	1.35	.080
Means				25.4	25.5	1.29	.079

n = average number of individuals analysed per locus
s = total # of populations at site
L = # of loci studied
P_{99} P_{95} = proportion of loci polymorphic, 99% and 95% criteria respectively
A = average # of alleles per locus
H_e = average expected heterozygosity within populations
*Rankin Inlet not included in ANOVA of heterozygosity

of undetected species complexes within those taxa with apparently broad distributions. Indeed, genetic studies may be the best, if not the only, method to examine the postglacial history of most zooplankton taxa because their thin cuticles do not readily fossilize.

Studies of actively dispersing terrestrial taxa have been unable to demonstrate a significant loss of variability between ancestral and derived populations (Bryant et al., 1981; Berlocher, 1984; Parkin and Cole, 1985; Baker and Moeed, 1987; St. Louis and Barlow, 1988; Easteal, 1985). This study demonstrates that pond copepods lose a significant amount of genetic variation at their polymorphic loci during colonization, probably due to their reliance on passive dispersal. This suggests that profound genetic changes in zooplankton taxa are possible due to colonization alone. Such changes in the nature and amount of genetic variation have been suggested as prerequisites to speciation (Mayr, 1982; Carson and Templeton, 1984). Copepods and other passively dispersed aquatic taxa may prove useful in future studies designed to evaluate these speciation theories. However, these investigations must await better genetic based taxonomy, at least in the copepods.

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Chapter V

Morphological and Ecological Variation in *Hesperodiaptomus arcticus*, a species complex

- Introduction

The taxonomy of many freshwater zooplankton groups remains unstable, as it is shown that the "species" recognized by classical systematists consist of a number of reproductively isolated taxa. These taxonomic problems remain unresolved in many groups despite a century or more of study employing traditional techniques. A a result it is increasingly being recognized by zooplankton systematists (Frey, 1982; Einsle, 1980, 1988; Bradford, 1988) that it is difficult to delimit species boundaries using morphological criteria alone.

The freshwater cyclopoid copepods have been recognized as a persistently difficult taxonomic group (Yeatman, 1944, 1959; Smith, 1981) but the North American calanoid copepods have received little taxonomic study for the last 30 years (Kincaid, 1953, 1956; Wilson, 1941, 1954, 1959) and few new species have been described over this period (e.g. Anderson and Fabris, 1970; Anderson, 1971a; Dussart and Defaye, 1983). This has led to perception that the existing calanoid taxonomy is satisfactory (Smith and Fernando, 1978; Pennak, 1978; Balcer et al. 1984) and such treatments suggest that many calanoids are morphologically variable and broadly distributed.

For example, *Hesperodiaptomus arcticus* is a large bodied calanoid copepod thought to be widespread (Wilson 1959)

in pond habitats from the Canadian Rockies (Reed, 1959; Anderson 1971b; 1974; Hooper, 1947) as far east as Ungava (Reed, 1959; J.C.H. Carter, personal communication) and from the prairies (Wilson, 1958) to the high arctic (Marsh 1929; Reed 1962, 1963; Tash, 1971). The first evidence of potential taxonomic complications was obtained from allozyme studies at Churchill, Manitoba which revealed that genotypic frequencies in many populations deviated from Hardy-Weinberg expectations due to the absence of heterozygotes at three enzyme loci (Chapter 4). Such heterozygote deficiencies are most readily explained if H. arcticus sensu lato at Churchill consists of two reproductively isolated taxa. Field and laboratory observations confirmed morphological and ecological differences between these two taxa. Subsequent allozyme studies over a broader geographic range revealed a third genetically distinct taxon.

The present study employed allozyme electrophoresis in integrated investigations of the morphology and ecology of these three members of the H. arcticus group. Morphological characters of Hesperodiaptomus arcticus s. l. were examined in electrophoretically classified and museum specimens to determine the characters that delimit these three taxa. A detailed study was also undertaken at Churchill to examine reproductive, life history traits and habitat differences between the two taxa that co-ocurred at this site.

Material and Methods

Collections and Electrophoretic Analysis

Live specimens were collected by net from 2-6 populations at 11 Canadian localities between July 1984 and August 1988 (Table 5-1; sites 1-11). Normally a portion of each sample was preserved immediately after collection for morphological analysis. Preserved material was obtained from three additional Canadian sites and from four sites in the United States (Table 5-1; sites 12-17). Three genetic isolates of H. arcticus (Isolates A, N and C) had previously been delimited on the basis of electrophoretic analysis of 20 gene loci (Chapter 4). Using electrophoretic criteria, live collections were classified to isolate type and populations shown to contain only single isolates were used for the morphological analysis.

Morphological Analysis

In order to ascertain the extent of morphological variation within each of the 3 isolates of the Hesperodiaptomus arcticus group, several body measurements and meristic counts were undertaken on 48 slide-mounted specimens of electrophoretically classified populations. The sexually dimorphic 5th legs were examined because these limbs are the usual basis of species diagnostic traits in all Diaptomidae. The segment proportions and setation patterns (nomenclature of Wilson, 1959) of the antennules were also determined from representative populations. Morphological differences evident between the genetic

Table 5-1

Locations of sites and numbers of populations studied for genetic, morphological and ecological traits of Hesperodiaptomus arcticus in North America. Letters were assigned to correspond to the isolates of H. arcticus (N=Isolate N, C=Isolate C, A=Isolate A). Museum specimens were classified based on morphology (see Results).

Sites #	Name	Isolate type	# Pop's	Description
1	Churchill, Manitoba	N C	42 27	58°47'N 94°11'W 15 km east of townsite near former NRC launch
2	Eskimo Point (Arviat) Northwest Territories Dist. of Keewatin	N	3	61°7'N 94°3'W within immediate vicinity of village site
3	Rankin Inlet, N.W.T. Dist. of Keewatin	N	3	62°49'N 92°5'W within immediate vicinity of townsite
4	Paulatuk, N.W.T Dist. of Mackenzie	N	2	69°22'N 124°2'W north of village
5	Tuktoyaktuk Peninsula, N.W.T. Dist. of Mackenzie	N	1	69°39'N 131°55'W on peninsula 60 km east of townsite
6	Nicholson Point, N.W.T. Dist. of Mackenzie	N	2	69°57'N 128°54'W Dew Line installation
7	Tuktoyaktuk, N.W.T. Dist. of Mackenzie	A	5	69°27'N 133°2'W within immediate vicinity of townsite
8	Mackenzie Delta, N.W.T. Dist. of Mackenzie	A	1	68°55'N 134°32'W eastern rim of delta near Tununuk Point
9	Banff National Park, Alberta	N	2	51°29'N 116°3'W Boulder Pass, 11 km east of Lake Louise
10	Jasper National Park, Alberta, Cavell Lake	N	1	52°42'N 118°4'W 19 km south of Jasper
11	Saskatoon, Saskatchewan	C	4	52°8'N 106°44' 2km east of Saskatoon

Table 5-1 cont'd

Sites #	Name	Isolate type	# Pop's	Description
12	Herschel Island, Yukon Territories	A	1	69°16'N 138°55'W (approx.) USNM Cat# 058793 syntypes (F. Johansen)
13	Ungava, Quebec	C	3	58°N 68°29'W (approx.) False River
14	Cape Thompson, Alaska Lake #6	A	1	69°12'N 165°35'W USNM Cat# 210304 (J. Tash)
15	Talkeetna, Alaska Ice Cake Lake	N	1	62°30'N 150°W (approx.) USNM Cat# 210303 vial #2 (M.S. Wilson)
16	Prince Albert, Saskatchewan	C	2	53°13'N 105°42'W near confluence of north and south branches of Saskatchewan River
17	Heart Butte, Montana Pondera Co.	C	1	49°N 113°15'W (approx) USNM Cat# 210303 vial #2 (M.S. Wilson)
18	Pilsbury, North Dakota	C	2	47°16'N 97°48'W between Valley City and Finley

isolates were then used as a basis for the classification of the preserved specimens. Body lengths (anterior margin of cephalosome to distal margin of caudal rami) were measured in 559 preserved specimens from populations over the entire known distribution of Hesperodiaptomus arcticus s. l.

Detailed Ecological Analysis at Churchill

In order to assess the distributions of isolates C and N a survey, using field identification characteristics only, was conducted in 1987. Carotenoid pigmentation was paler, and the antennules were shorter in Isolate C than in Isolate N. Populations in 50 ponds on Rock Bluff C were recorded as possessing either or both isolates. A subset of 18 ponds from this survey was removed to the laboratory and 24 animals electrophoresed for three diagnostic enzymes (GOT, MPI and PEP-C) to evaluate the accuracy of the field identifications. The presence of faster electromorphs for MPI and PEP-C and slower electromorphs for GOT-1 confirmed the presence of Isolate C, while slower electromorphs for MPI and PEP-C and faster electromorphs for GOT-1 confirmed the presence of Isolate N. A mixture of 2 genotypic combinations at these respective loci confirmed the presence of both isolates. To verify the allelic assignments four individuals were run on each gel from ponds inhabited by single isolates (C90 -- isolate C, A21 -- isolate N).

During July and August 1988, a study of the maturation and reproductive phenology was conducted on 4 populations of each isolate from ponds on Rock Bluff C. Two of these four

populations contained a single isolate (C90, C89 -- isolate C; C44, C45 -- isolate N) and two populations contained both isolates (C51 and C156) so that a total of 6 ponds were surveyed. Ponds were sampled weekly with a net and the sample divided into 4 equal subsamples. Animals from one subsample were anesthetised with carbonated water and immatures, mature males, non-reproductive and reproductive females were counted. Reproductive females were those which possessed either an egg sac, a spermatophore, or mature ova in their ovaries. Anesthetization was used because the mature ova in the ovaries are difficult to observe and females often lose their egg sacs when placed in preservatives such as formalin or ethanol. Additional subsamples were counted as necessary to obtain a minimum of 100 animals for each isolate present in a population. This method of subdividing the net samples was necessary because the abundances of the isolates were often significantly unequal in ponds where they coexisted. When the minimum sample size was achieved for the more abundant isolate, it was not enumerated in any other subsamples, which were counted to achieve the minimum sample size for the less abundant isolate. Because the sample sizes were different for each isolate all counts were converted to proportions.

The permanence of habitats occupied by the two isolates was examined using an independent index of ephemerality determined for a large number of ponds at the Churchill site during 1984 and 1985 (N. Billington unpublished data).

Ponds were monitored weekly beginning in June and determined to have water or to be dry. The index was the week after the original sampling in which the pond went dry or the week of the final observation (week 13 in 1984; week 14 in 1985). Only 50 ponds on Rock Bluff C (Appendix VI), where the 2 isolates coexisted, were used in this comparison.

Results

Electrophoretic Classification of H. arcticus s. l.

Isolate A was collected least frequently, being found in only 5 ponds in the immediate vicinity of Tuktoyaktuk and in 1 pond from the Mackenzie Delta (Table 5-1). Isolate C was present in 31 ponds at Churchill and Saskatoon, while Isolate N was found in 56 ponds at 8 sites.

Morphological Correlations

Females of all isolates possessed three segmented urosomes (Figure 5-1a-c). The genital segments were ordinarily expanded laterally, but this character was extremely variable even within isolates from different localities. On the other hand males for all 3 isolates possessed 5 urosomal segments (Figure 5-1d) which did not vary in shape or ornamentation.

The antennules of each species were composed of 25 segments. The pattern of major antennule setae was invariant between sexes and taxa and conformed to the pattern for most Hesperodiaptomus (Table 5-2). However, there were differences in major setae lengths, segment 1 setules and in the shape of the antennular segments. Figure

Figure 5-1

Body shapes of female and male Hesperodiaptomus arcticus s. l. isolates. a-Isolate A female; b-Isolate N female; c-Isolate C female; d-Isolate N male. Scale bar=1 mm.

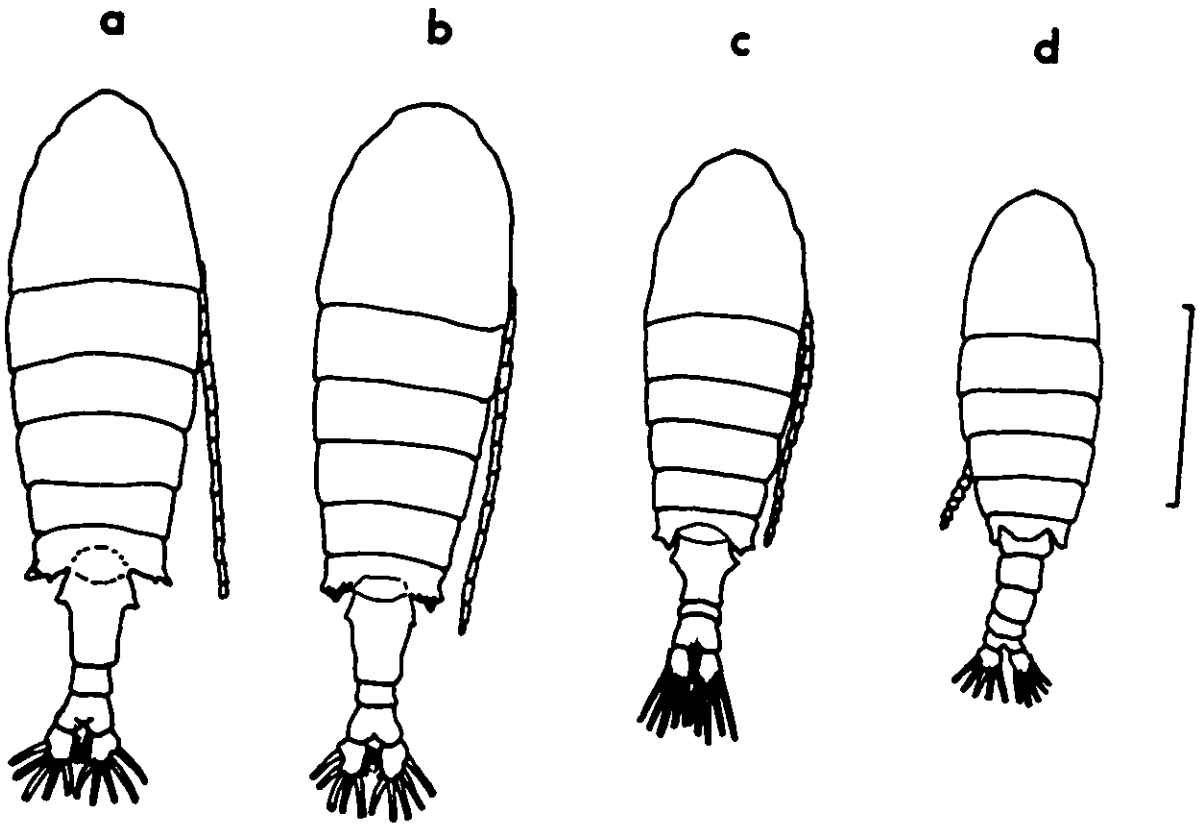


Table 5-2

Comparison of antennule setation pattern in 3 Hesperodiaptomus arcticus s. l. isolates. Pattern observed in females or left side of males. (s=seta; sp=spine; a=aesthete[1])

Segment	H. arcticus A	C	N
1	s,a	s,a	s,a
2*	3s,a	3s,a	3s,a
3	s,a	s,a	s,a
4	s	s	s
5	s,a	s,a	s,a
6*	s	s	s
7	s,a	s,a	s,a
8	s,sp	s,sp	s,sp
9	2s,a	2s,a	2s,a
10*	s	s	s
11*	2s	2s	2s
12	s,sp,a	s,sp,a	s,sp,a
13*	s	s	s
14*	s,a	s,a	s,a
15*	s	s	s
16*	s,a	s,a	s,a
17,18*	s	s	s
19*	s,a	s,a	s,a
20	s	s	s
21-24	2s	2s	2s
25	5s,a	5s,a	5s,a

1-small club shaped elongation, see Figure 5-2.
*-segments where variation in pattern among Hesperodiaptomus species has been noted.

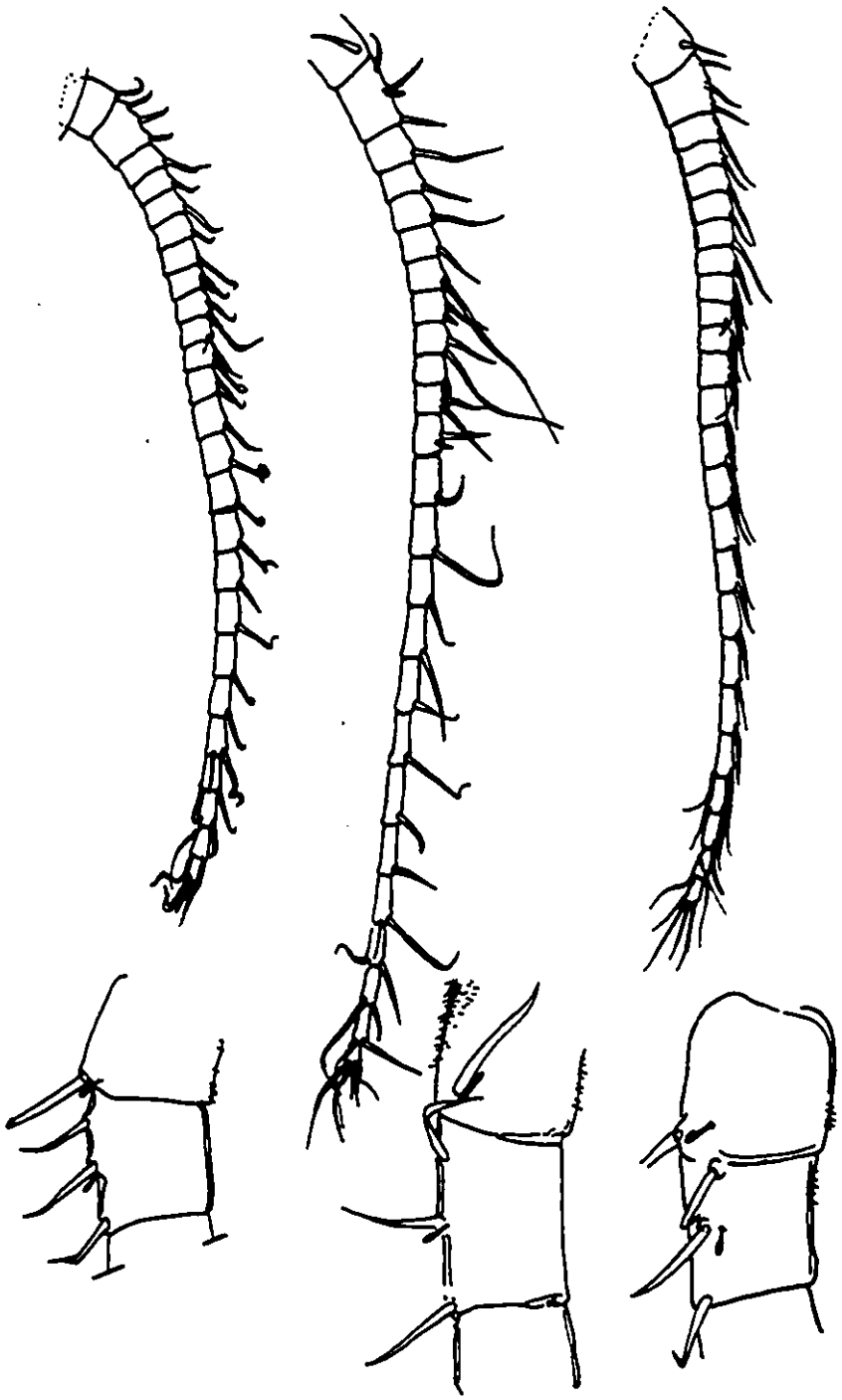
5-2 illustrates two characters useful in the discrimination of females of isolate N from those of isolates A and C. Setae on segments 7 and 9 are extremely long in the isolate N, reaching to segment 14 (Figure 5-2b), whereas these setae are short, not reaching past segment 12 (Figure 5-2a,c) in Isolates A and C. Very fine setules are also present on the anterior margin of antennule segment 1 in Isolate N, but absent in the other 2 isolates. Similar relative differences in the lengths of the major setae exist in males of these isolates, but the difference is not as clear. However, the setules on segment 1 are also present in Isolate N males but absent in males of the other two isolates.

Isolate N also differed from the other two isolates, in that the antennule reached to the middle of the genital segment of the urosome (Figure 5-1b), whereas it did not usually reach past the distal margin of the metasome in the other two isolates. This characteristic was readily visible in live animals and was used in field identifications. The length to width ratio in antennule segments was always greater in isolate N and this was especially evident in segment 2 (Figure 5-2). Specifically, the ratio of length to width in segment 2 was always greater than 1.0 in isolate N but less than 1.0 in isolates A and C.

Isolate A was always distinguishable from those of Isolates C and N by the morphology of the spinules on the external margin of the terminal spine of the 3rd exopod segment of the 4th leg (Exp_3P_4). Terminal spines on Exp_3P_4

Figure 5-2

Setation of antennules in female H. arcticus s. l. isolates with details of segment 1 respectively. a-Isolate A; b-Isolate N; c-Isolate C. Aesthetes not shown on all segments.



in isolate A possessed only 15-20 stout spinules on the external margin and internal margin setules were either sparse or absent (Figure 5-3a,b) in females and males. By contrast individuals belonging to isolates N and C possessed finer spinules on the external margin which graded into setules apically. In addition setules were always present and dense on the internal margin (Figure 5-3c-d).

The sexually dimorphic 5th legs were variable in males even within isolates. The sclerotized structure of the right basal segment 2 (Figure 5-4a-c) did not distinguish the isolates. The teeth in the margin of this structure varied from laminar (Figure 5-4a) to conical (Figure 5-4c) and were arranged in a row or scattered. The symmetric legs of females were not as variable within or between isolates but could not be used to distinguish the isolates (Figure 5-4d).

Using the morphological differences between the three isolates a dichotomous key to the taxa was constructed and the remaining preserved specimens were examined and identified:

1. Terminal spine on exopod 3, of leg 4 with 15-20 stout spinules only on external margin, internal margin with or without sparse setules.....Isolate A

   Terminal spine on exopod 3, of leg 4 with >20 fine spinules grading into fine setules on external margin, internal margin with numerous setules.....2

2. Antennule segment 2, length > width; segment 1 with setules on anterior margin; setae on segments 7 and 9 long in females, extending to segment 14.....Isolate N

   Antennule segment 2, length $\leq$ width; segment 1 without setules; setae on segments 7 and 9 short in females; never extending to segment 14.....Isolate C

Figure 5-3

Terminal spines of Exp_3P_4 in H. arcticus s. l.
a-Isolate A female, b-Isolate A male, c-Isolate N female, d-Isolate C female. Internal margin of spine to the right. Scale bar=.001mm.

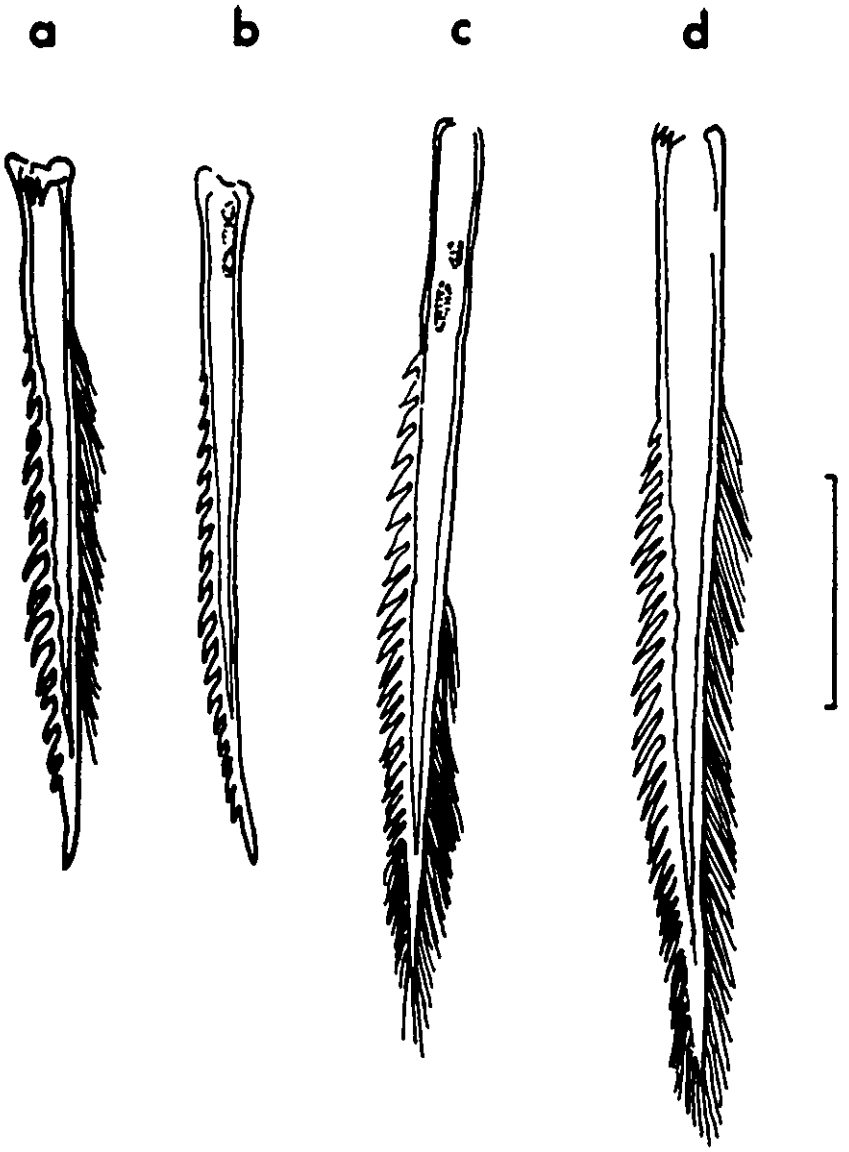
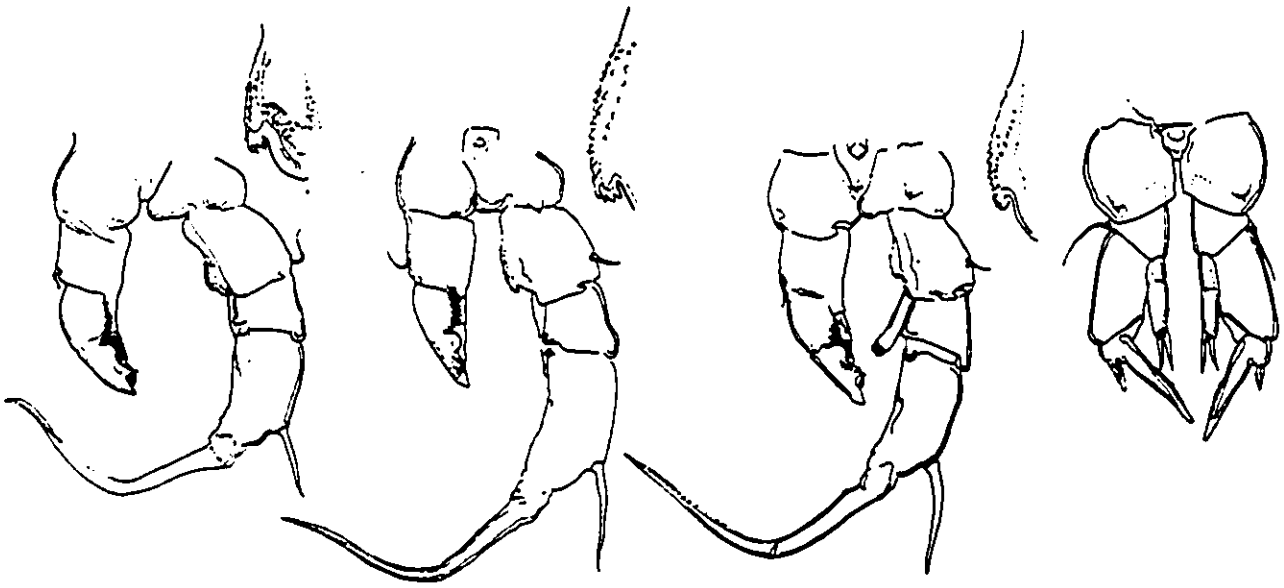


Figure 5-4

Sexually dimorphic fifth legs of H. arcticus s. l.
a-Isolate A male, b-Isolate N male, c-Isolate C male,
d-Isolate C female. All figures viewed dorsally.



Of the seven sites classified using these criteria (Table 5-1) two sites were identified as Isolate A (Herschel Is., Cape Thompson), one site as Isolate N (Talkeetna) and the four remaining sites as Isolate C.

Following identification, adult body sizes of 559 individuals were determined from 21 populations at 14 sites (Table 5-3). These analyses showed that females were invariably larger than males from the same location. There was a clear link between body size and latitude, with the largest animals in southern latitudes. Thus, the largest individuals found were from a prairie sample of Isolate C (North Dakota) where the average for males was larger than the average females from all other sites. The smallest individuals were obtained from Herschel Is. (Isolate A) the most northerly of the sample localities. These individuals were half as large as prairie individuals. A similar pattern was seen within isolates although Isolate N animals from the 3 high altitude ponds (1500-2300 m asl) of Banff and Jasper National Parks were similar to those from the low arctic at Churchill. The two isolates which co-occurred at Churchill were of similar size.

Ecological Characteristics of Isolates C and N

The survey of Rock Bluff C showed that Isolates C and N were distributed over the entire bluff (Figure 5-5) in approximately equal frequency. Of 50 ponds visually surveyed in 1987, 20 contained only isolate C and 23 only isolate N. The two taxa co-ocurred in seven ponds.

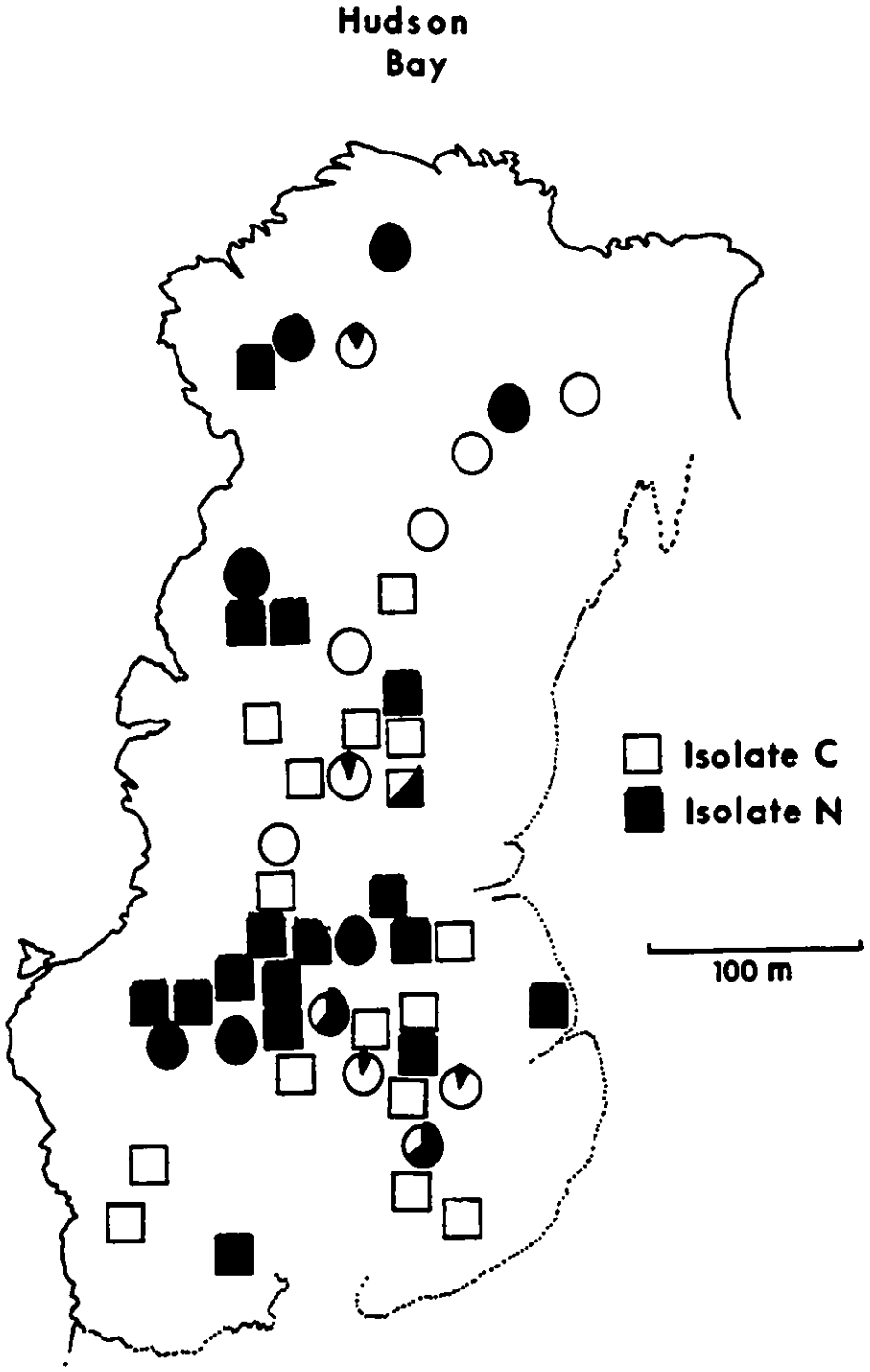
Table 5-3

Adult body sizes (mm ± SE) of *Hesperodiaptomus arcticus* isolates from 13 sites (site descriptions in Table 5-1). Numbers in parentheses are sample sizes (f=females; m=males).

Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
		Isolate A			
Herschel Is.	Tuktoyaktuk	Cape Thompson	------	------	------
(25f) 2.58±.02	(10f) 3.20±.04	(13f) 2.72±.02			
(10m) 2.49±.03	(4m) 3.03±.06	(11m) 2.59±.03			
	(19f) 3.18±.03				
	(4m) 2.99±.07				
	(15f) 3.50±.04				
	(1m) 2.76				
		Isolate M			
Paulatuk	Talkeetna	Churchill	Jasper	Banff	------
(7f) 3.06±.04	(23f) 2.94±.03	(6f) 3.34±.08	(7f) 3.51±.06	(25f) 3.38±.03	
(1m) 2.99	(25m) 2.72±.04	(6m) 3.17±.05	(10m) 3.34±.03	(14m) 3.25±.05	
		(5f) 3.38±.04		(28f) 3.18±.03	
		(3m) 2.98±.03		(0m) ----	
		(15f) 3.51±.04			
		(20m) 3.38±.03			
		Isolate C			
Churchill	Ungava	Saskatoon	Prince Albert	Heart Butte	Pilsbury
(21f) 3.14±.04	(6f) 3.04±.06	(2f) 3.27±.03	(6f) 3.56±.12	(25f) 4.44±.04	(3f) 4.50±.07
(9m) 2.95±.05	(1m) 2.84	(4m) 3.18±.12	(6m) 3.38±.03	(7m) 3.76±.10	(14m) 3.81±.04
(20f) 3.04+.04			(5f) 4.38±.10		
(20m) 2.88+.04			(7m) 3.78±.13		

Figure 5-5

Distribution of Isolate C and Isolate N on Rock Bluff C. Circles denote populations where proportions were determined by electrophoresis of a random sample. Squares denote field determinations only.



Electrophoretic analysis of individuals from 18 ponds confirmed all populations were correctly identified, although in one instance a small percentage of Isolate N was found in a pond judged to include only Isolate C.

There were significant differences (Mann-Whitney U test) in the ephemerality index of ponds containing the two isolates (Figure 5-6). Few ponds that dried out in 6 weeks or less were inhabited by isolate N and the majority of ponds persisted for 10 or more weeks. On the other hand isolate C was common in ponds that dried in fewer than 6 weeks and 67% of the populations were found in ponds which did not persist beyond 10 weeks of the season.

Results of the temporal abundances of immatures and reproductive females of the two isolates revealed an approximate two week delay in maturation (Figure 5-7) and reproductive activity (Figure 5-8) for isolate N. Two ponds dried out prior to the completion of the observations (C90, August 5th; C51, July 29th). Isolate C had reached 100% mature females in the both populations (Figure 5-8a) but isolate N had not in C51 (Figure 5-8b).

Discussion

Diaptomid copepods have long been considered a difficult group owing to their morphological variability (deGuerne and Richard, 1889: Schacht, 1897), and the large bodied "*shoshone*-group" (Wilson, 1953; Kincaid, 1953; 1956) has been a difficult assemblage. *Hesperodiaptomus arcticus*, a large

Figure 5-6

Distribution of persistence durations (E-ephemerality defined in text) for Isolate C and Isolate N. Abscissa represent proportion of 27 Isolate C (open bars) and 30 Isolate N (solid bars) populations in each E class.

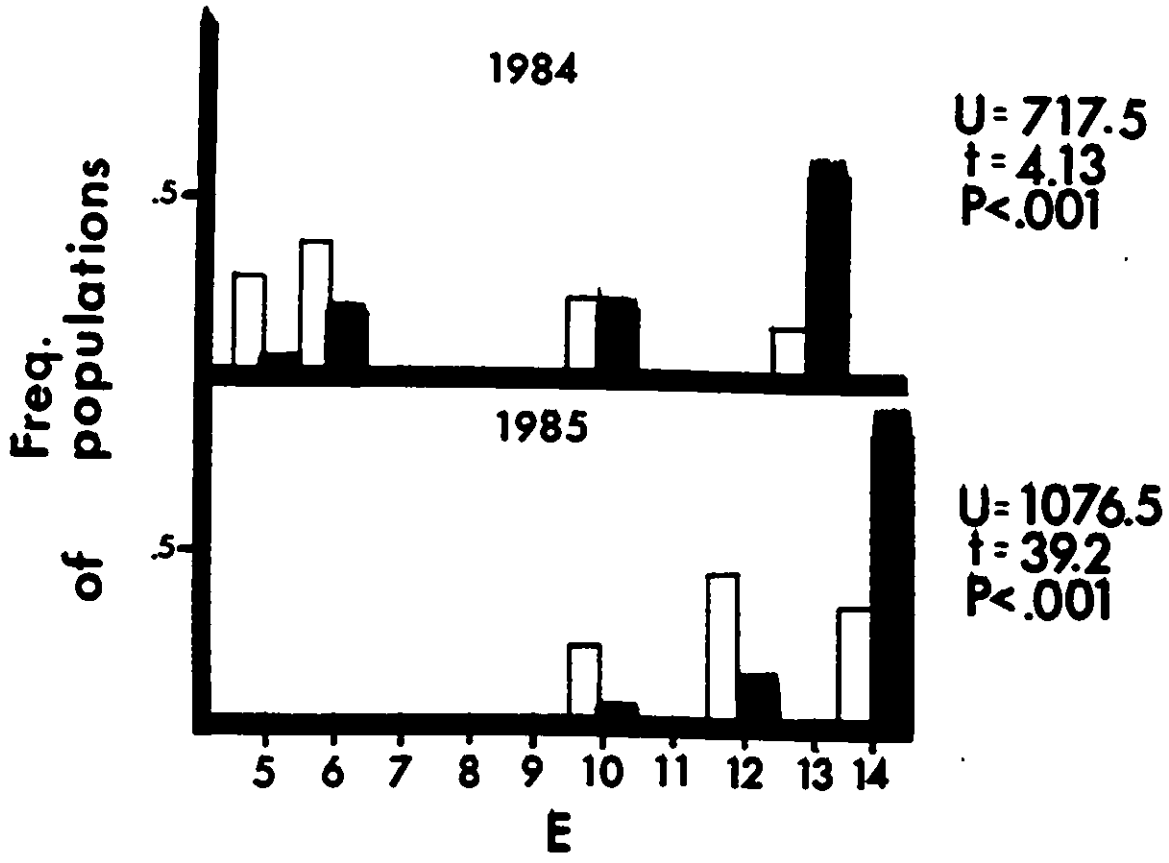


Figure 5-7

Proportions of all individuals which were immature, over time, in two Isolate C and two Isolate N populations. Each profile represents the proportion one population where single isolates occur.

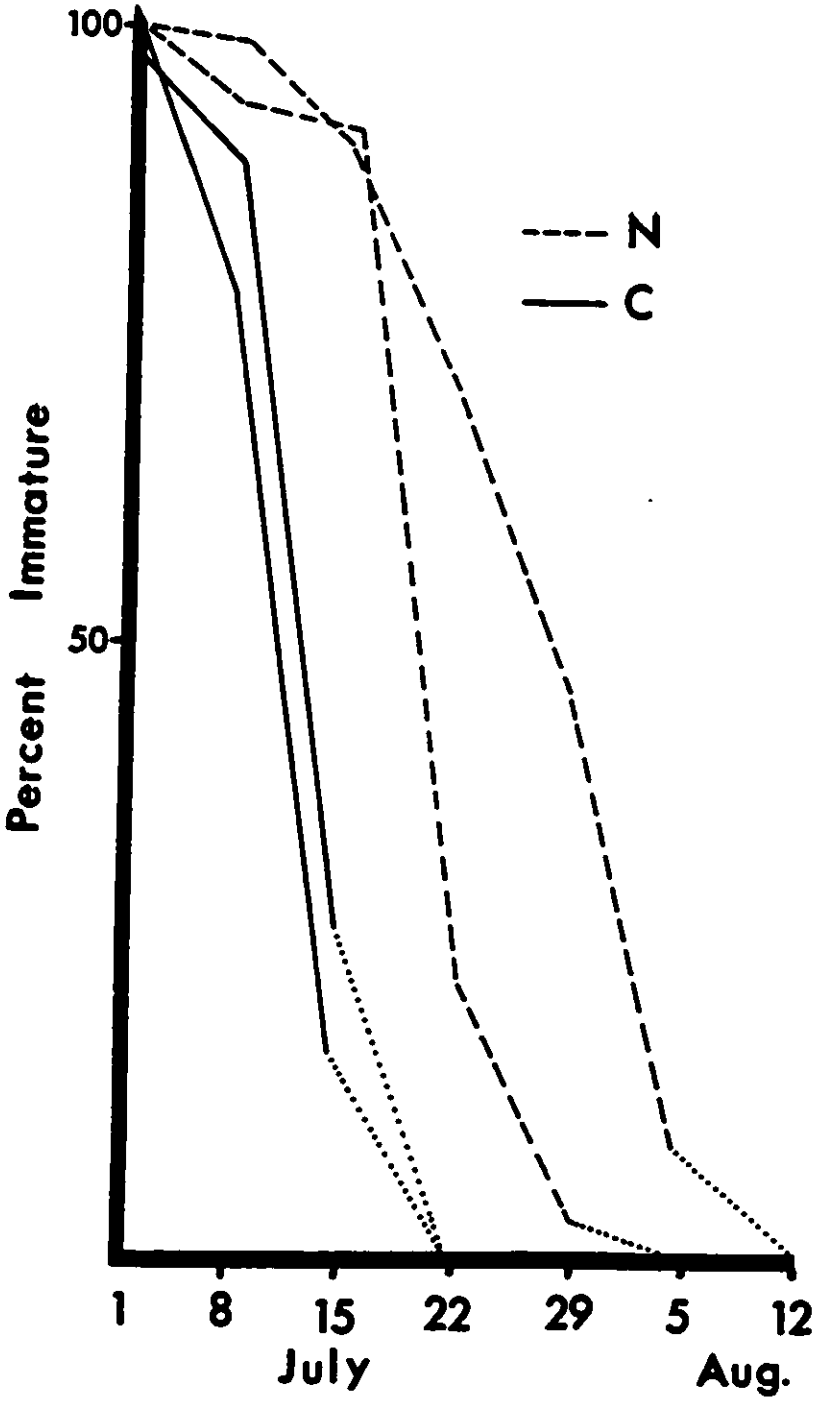
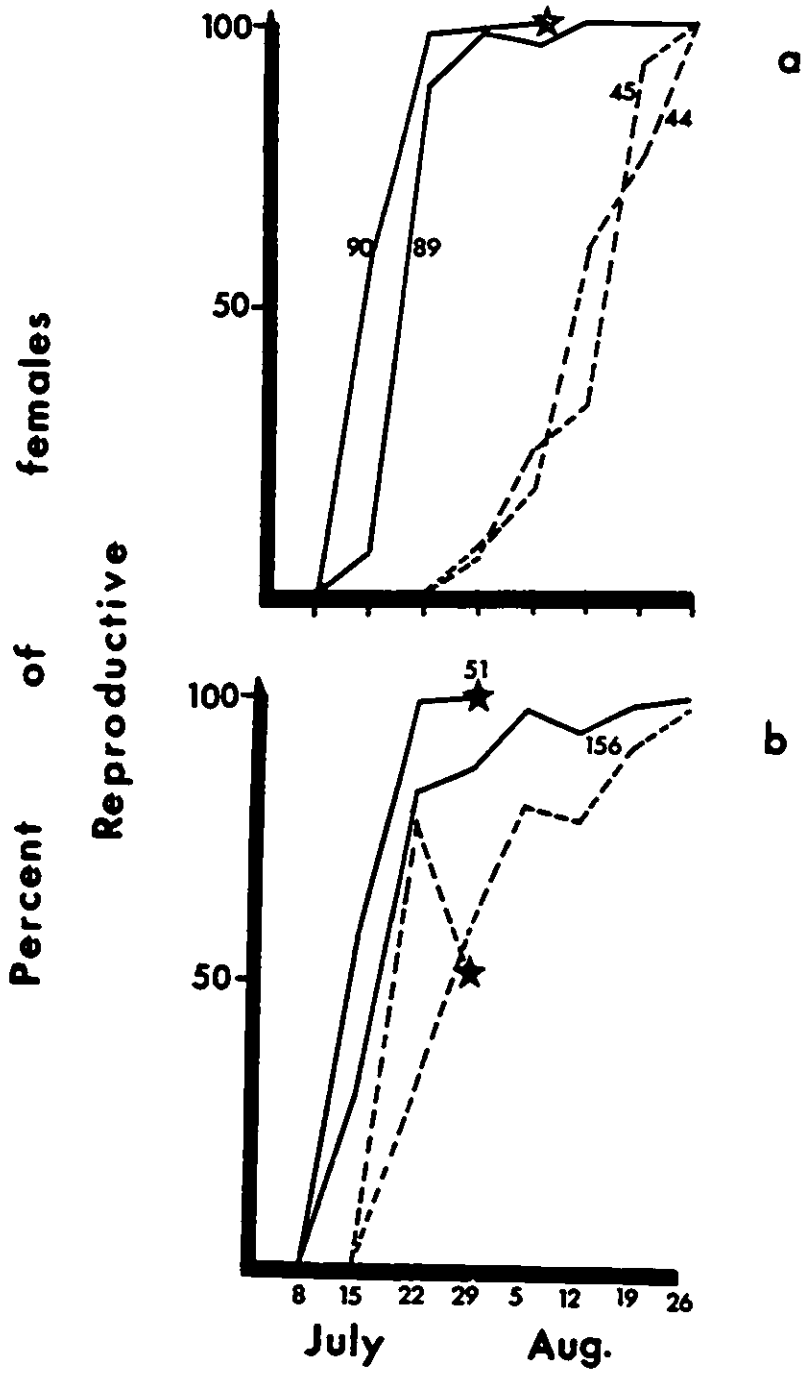


Figure 5-8

Proportion of females which were in reproductive state from six Isolate C and Isolate N populations. In two Isolate C and two Isolate N populations, the isolates occurred alone (a) and in two populations the isolates co-occurred (b). Stars denote dates on which ponds no longer contained water.



bodied pond calanoid was described as "closely related to...shoshone" by Marsh (1920, p.73) from specimens collected on Herschel Island, Yukon. The species was primarily recognized by the lateral expansions of the urosome in females and the cuticular apparatus of basal segment 2 on the fifth leg in the males. Subsequently the species was identified in arctic and alpine habitats throughout North America. The present study has shown that these characters are variable within H. arcticus s. l. and such variation has probably contributed to early confusion with another large copepod, H. eiseni (Brewer, 1898; Bajkov, 1929; Rylov, 1930; Carl, 1940; Kincaid, 1956). Despite such variation the distinctiveness of H. arcticus has never been challenged (Wilson, 1959). The present studies have shown clearly, however, that Hesperodiaptomus arcticus is not a single species.

Three taxa, which are genetically different enough to warrant recognition as distinct species, can also be distinguished morphologically. The specimens from the type locality at Herschel Island possessed the same characters that distinguished the electrophoretically classified Tuktoyaktuk and Mackenzie delta specimens. Two new species are proposed (Hesperodiaptomus churchillensis=Isolate C and Hesperodiaptomus nearcticus=Isolate N), which can be distinguished from specimens of the type species Hesperodiaptomus arcticus sensu stricto by the possession of finely spinulated terminal spines on the exopod of the 4th swimming feet (Figure 5-4). Hesperodiaptomus churchillensis

is further distinguished from H. nearcticus by the dimensions of antennular segment 2 and by the setae length on antennular segments 7 and 9.

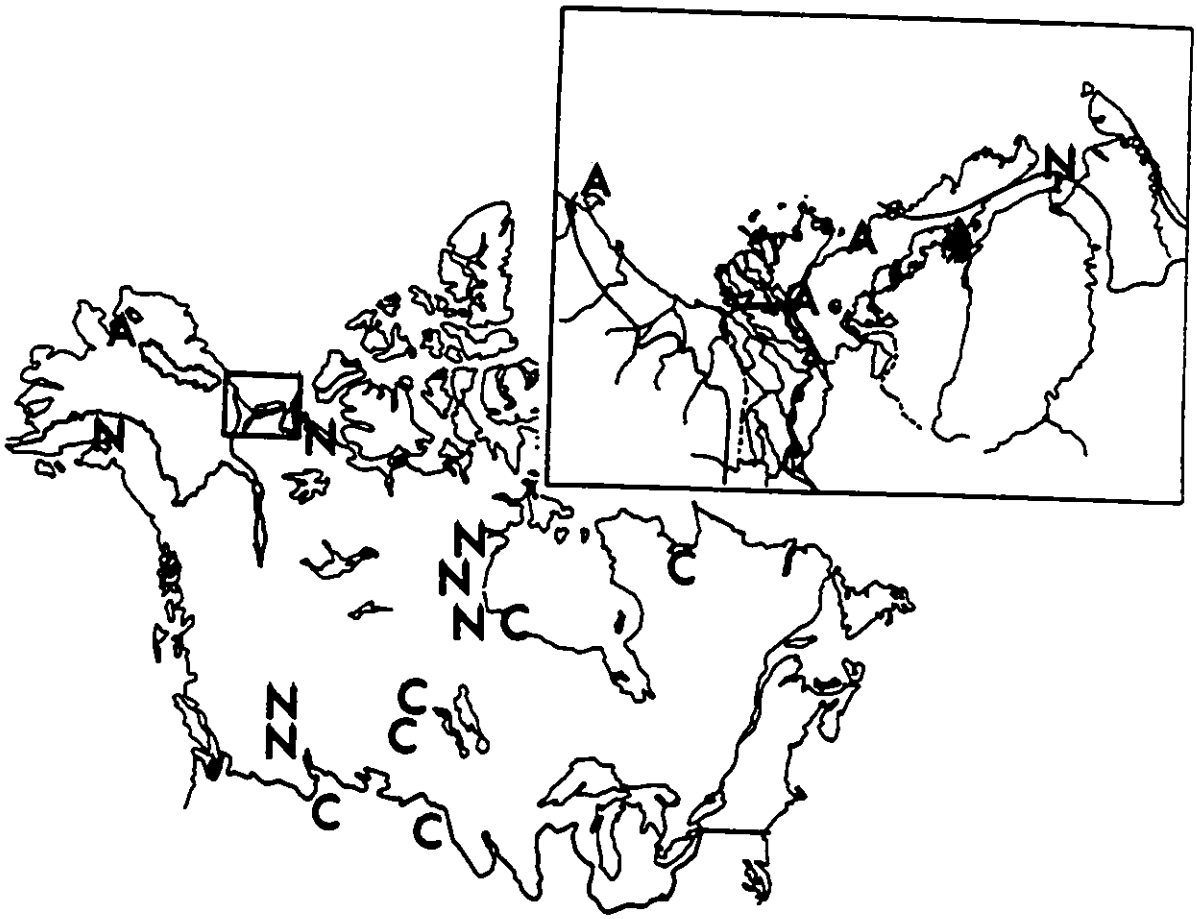
The three species have largely allopatric distributions in North America (Figure 5-9) although H. churchillensis and H. nearcticus co-ocurr at Churchill, Manitoba. The latter two species generally show microallopatry, with H. churchillensis inhabiting more ephemeral ponds.

Hesperodiaptomus arcticus sensu stricto appears to be restricted to the western arctic areas in the immediate vicinity of the Beringian refuge (Figure 5-9). The species has apparently failed to colonize areas which became deglaciated, while the other two species have expanded extensively from their refugia. The distribution of H. nearcticus in sites near cordilleran and arctic refugia suggests it could have colonized from either or both regions. Further genetic study at these localities will be needed to distinguish between these two possibilities. On the other hand H. churchillensis is distributed from the prairies to the treeline, as far east as Ungava Bay (Figure 5-9), which suggests that the species survived maximum glaciation south of the continental ice sheet. This conclusion is also consistent with those of an allozyme genetic study (Chapter 4) which shows that southern populations (Saskatoon) of this species possess more variation than those in the north (Churchill).

The role of Pleistocene glaciations has been a focal point of biogeographical studies in North America (Fernald,

Figure 5-9

Distribution in North America of three Hesperodiaptomus species, including two new species. All localities confirmed in this study only are illustrated. A-H. arcticus s.s.; N-H. nearcticus; C-H. churchillensis.



1925; Carpenter, 1928). Aquatic organisms which populated most of Canadian North America, colonized the region from founder stocks located in areas that were unglaciated. Existing distributions have been used to infer past colonization histories (Ross, 1965; Dadswell, 1974). However, the use of distributional information in most zooplanktonic taxa relies on morphology-based systematics, which is inadequate (Frey, 1965, 1982) even in the calanoid copepods that had been considered systematically stable. The present study has demonstrated that Hesperodiaptomus arcticus s. l. is composed of three morphologically distinct species, with different postglacial histories. There is a proven need for further genetically based studies in copepods because traditional techniques, alone, are unable to provide reliable delimitation of species. Indeed, where biogeographical studies propose to infer evolutionary patterns, genetics studies are likely the only useful approach, as they alone provide unambiguous information on reproductive isolation.

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Chapter VI

General Discussion

Although the copepod crustaceans have been studied for more than 200 years, little is known about their genetics and the freshwater Copepoda have always been less intensely studied than their marine relatives. The aim of this investigation was to characterize the level and nature of genetically determined biochemical variation in freshwater Copepoda. The results necessitated an examination of the systematics within one calanoid group and suggested a number of areas for future investigation. For example, biogeographical studies that attempt to discern evolutionary relationships (phylogenies) from distributional patterns depend upon stable taxonomy.

The present genetic studies have shown that there is reason to doubt the systematic validity of some freshwater copepod taxa, which were thought to have a continental distribution. Therefore, freshwater copepod taxa that are global in distribution must be considered cautiously, as it is uncertain whether any such taxa exist as genetically cohesive species. As well, pond copepod gene frequencies were significantly differentiated among extremely localized populations, which is contrary to the widely accepted notion that many taxa are widespread due to their efficient dispersal capabilities. Indeed, the genetic differentiation was not consistent with probable dispersal capabilities as predicted by equilibrium population genetics theory, but

rather was a measure of founder numbers due to the young age of populations. It is evident from these results that there is a need among copepodologists to clarify the systematics of the Copepoda. However, once such taxonomic studies are complete, copepods should prove to be excellent for studies designed to evaluate theoretical evolutionary models of genetic differentiation and speciation.

Biochemical Systematics in Copepoda

The taxonomy of most copepod groups has remained unstable despite two centuries of study employing traditional techniques. It is clearly difficult to delimit species boundaries using morphological criteria alone, but integrated applications of morphological and other techniques (Einsle, 1980, 1988) are more successful. The present studies (Bolleau and Hebert, 1988; Chapters 4 and 5) have amply demonstrated that enzyme electrophoresis can be used to identify reproductively isolated copepod taxa.

Where copepods are broadly distributed, that is, cosmopolitan and even continental, the taxonomic assignments should not be considered reliable until genetic studies can confirm them. The complexity of the Hesperodiaptomus arcticus group was revealed by the present study and many similar cases undoubtedly remain to be discovered. Arctodiaptomus bacillifer, for example, has been examined morphologically by Kiefer (1971) in Europe and shown to be composed of 2 morphologically distinct species occupying different habitats, but the assignment of Canadian arctic

specimens to a single taxon (Marsh, 1920) has never been challenged.

Due to the sensitivity of cellulose acetate gel electrophoresis, allozyme analysis can be conducted on copepod species previously too small (e.g. cyclopoids, Chapter 2) for analysis. The cyclopoids, in particular members of Acanthocyclops, have been persistently difficult to delimit (Marsh, 1910; Lowndes, 1928; Aycock, 1942; Yeatman, 1944, 1959; Price, 1955, 1958; Smith, 1981). Allozyme analysis of Acanthocyclops vernalis sensu lato (Yeatman, 1959) from Essex and Kent counties revealed three genetically distinct pond, and one lake dwelling taxa (Appendix I). Studies are currently in progress on individual animals from this group which have been analysed electrophoretically without destruction of their cuticle so that subsequent morphological analysis can be completed. Studies of this kind have enormous potential, especially among the copepods, to resolve long-standing systematic uncertainties (Einsle, 1988).

Allozyme analysis can also be used in broader taxonomic surveys to examine the genetic relatedness of supraspecific taxa (Avise, 1974; Avise and Aquadro, 1983; Thorpe, 1983). European copepod systematists (Kiefer, 1932, 1978; Dussart and Defaye, 1983) have questioned the validity of recognizing only a single morphologically varied genus, Diaptomus sensu lato, and delimited several morphologically distinct genera and subgenera. However, many North American researchers have simply continued to group all species in

the single genus Diaptomus s. l. There are many similar cases where genetic boundaries remain unclear. Such conflicts can be resolved by genetic studies which ascertain whether taxa comprising a single large genus are subdivided into a number of genetically related groups.

My own analysis of 5 diaptomid species, including Hesperodiaptomus victoriaensis, at 20 allozyme loci reveals that genetic identities between this species and other hesperodiaptomids (Figure 6-1) is higher (mean I=0.61, range 0.44-0.69) than with Leptodiaptomus tyrrelli (0.30, range 0.29-0.31). Although comparisons have not been completed with other genera within Diaptomus s. l. these data are not consistent with Anderson's (1967) hypothesis of removing H. victoriaensis from the hesperodiaptomids. Furthermore, the distribution of single locus genetic identities (Figure 6-2) between Hesperodiaptomus sensu stricto and Leptodiaptomus s.s. is reverse J-shaped with approximately 65% of loci fixed for alternate alleles (i.e. I=0.0) in each taxon. Genetic identities between two decapod crustacean genera (I=0.41-0.74, Hedgecock et al. 1982) and Thorpe's (1983) I=0.2-0.3 for congeners suggest that recognition of Leptodiaptomus and Hesperodiaptomus as distinct genera is reasonable, but the genetic affinities of other diaptomids is in need of further study.

Genetic comparisons of higher order taxa have been conducted on vertebrates (Avise and Aquadro, 1983) and such

Figure 6-1

Cluster analysis (UPGMA) of Nei's unbiased genetic identities at 20 enzyme loci in one Leptodiaptomus and four Hesperodiaptomus species from 13 sites in northern Canada. (Lt-L. tyrrelli; Hv-H. victoriaensis; Hc-H. churchillensis; Hn-H. nearcticus; Ha-H. arcticus)

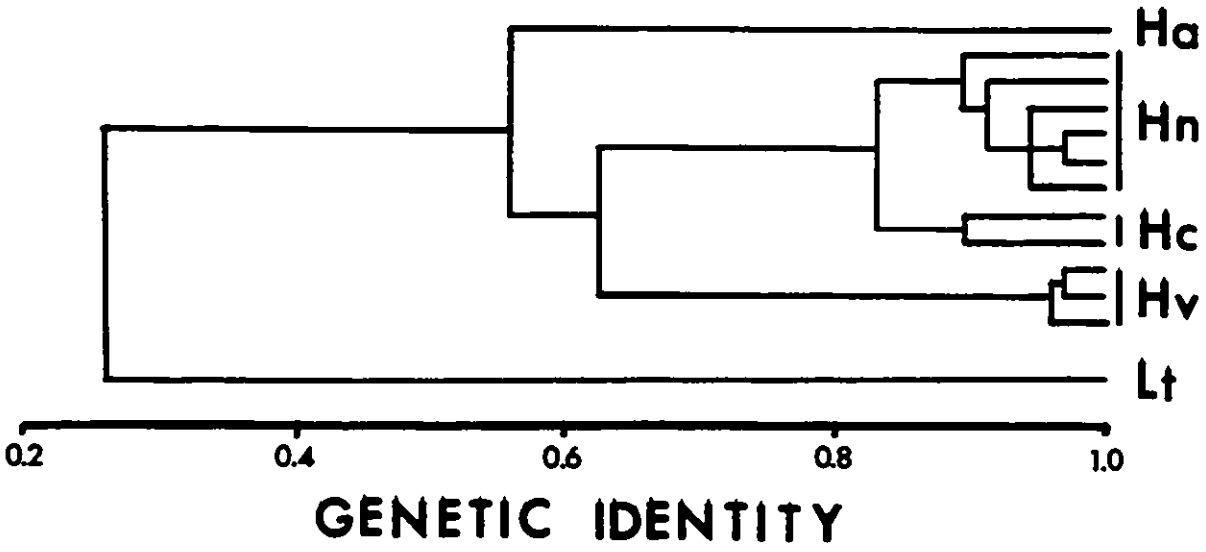
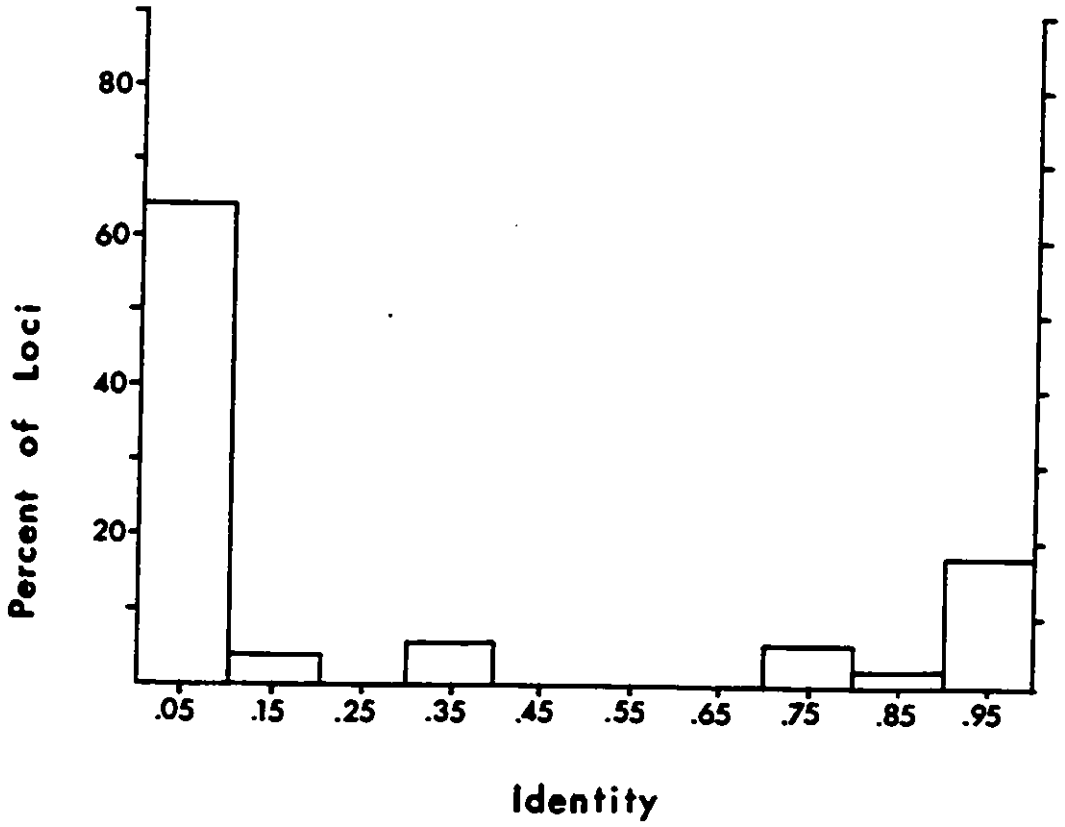


Figure 6-2

Percentage of 540 single locus coefficients of Nei's unbiased genetic identity between one Leptodiaptomus and four Hesperodiaptomus species.



comparisons have shown that the extent of genetic divergence differs among the vertebrate groups due to different taxonomic conventions. Therefore at present, it is inappropriate to consider the levels of genetic divergence among a few genera typical for crustaceans, or even copepods. More studies which compare such divergences, however, may provide the much needed independent criteria that will clarify systematic affinities within the copepods.

Distributions in Copepoda

Early attempts to examine the patterns in distribution of zooplankton in relation to North American glaciation (Brooks, 1957; Reed, 1959; Frey, 1965) have not been reexamined, although these studies are based on outdated systematics and geologic information (Flint, 1957). Some groups can be examined in conjunction with fossil evidence (cladocerans, ostracodes, notostracans), but genetic variation may provide the only means to examine phylogenies of copepod populations that have resulted from events such as glacial isolation and subsequent postglacial dispersal.

This study has revealed that the arctic pond copepods were not confined solely to a zone along the southern margin of the continental ice sheet during the Pleistocene, but rather that some species survived in Beringian areas (Heterocope septentrionalis, Hesperodiaptomus arcticus sensu stricto), and some may have persisted in unglaciated areas of the present arctic islands (H. nearcticus) such as Banks Island. Although the consideration of Banks Island as a

glacial refugium for H. nearcticus is speculative, the hypothesis would be supported if genetic variation within populations in this region was higher than those from the present study in glaciated locations.

Although the climate in Beringia is believed to have been harsh and supported only tundra (Colinvaux, 1963, 1964) many truly arctic copepods may have survived in this region (Hesperodiaptomus arcticus, H. nearcticus, Heterocope septentrionalis, Leptodiaptomus pribilofensis), while temperate and alpine taxa were confined to southern refugia (L. tyrrelli, H. churchillensis).

A contrast is visible in the distributions of two related arctic species. Hesperodiaptomus arcticus sensu stricto has apparently not become established beyond the margins of the late Wisconsin maximum ice position, while H. nearcticus has dispersed and become established as far east as Hudson Bay. Several other arctic species (Mixodiaptomus theeli, Nordodiaptomus alaskaensis, Diaptomus glacialis, Eudiaptomus gracilis) also appear not to have become established outside the immediate areas of western arctic ice boundaries. Species with similarly broad tolerences might show different distributional patterns (Martin and Chapman, 1965; Dadswell, 1974) due to varied dispersal capacities (Hebert and Hann, 1985), but it is hard to imagine that H. arcticus and H. nearcticus could differ much in this trait. This suggests that a re-examination of physical and ecological tolerences may be necessary in some copepod taxa in order to understand the present day distributions. Ross

(1965), for example, determined that the leveling of topographical features due to glaciation produced subtle physical conditions in streams that prevented certain insect species from recolonizing such areas and undetected limiting conditions could also curtail the copepods.

Dispersal in Zooplankton and Founder Effects to Genetic Variation

The present genetic studies confirm previous biogeographical information which suggests that copepod distributions are dispersal limited. Evidence from the distribution of gene frequencies among populations suggest that populations become founded by a few individuals. Once established, populations can expand rapidly to large sizes, and due to the infrequent movement of propagules, the genetic signature of the original founders can persist for many thousands of generations. The shift in gene frequencies to their equilibrium values is further slowed by the egg pool from which populations are refounded each year. Eggs that tend to remain dormant for many years cause overlapping generations and retard the decay of gene frequency differences among populations.

Discrete habitats may be colonized by a few populations which become a center of origin for other populations within the locality reflecting a genetic signature of this original "seed" population. Such extreme genetic differentiation of natural populations due to founder effects has largely been based on conjecture and theory, but the importance of

founder effects in copepod populations seems clear on the basis of allozyme data.

Further insight into the importance of founder effects may be gained by the examination of mitchondrial DNA (mtDNA) variation. Specifically, populations established from a few founder individuals should possess a small subset of the regional diversity of mtDNA types, which are maternally inherited. On the other hand, populations which have been founded either by large numbers of individuals and/or are frequently receiving many immigrants would possess a largely homogeneous array of the mtDNA types.

Rapid speciation is possible, in theory, when populations are founded by a few individuals (Bush, 1982) and if confirmed among copepods may provide insights into not only copepod evolution, but also speciation by the founder principle. Copepod speciation rates might be high among taxa which persist in dicrete isolated populations, but a more comprehensive understanding of their systematics will be required before this can be evaluated.

The present studies have shown that profound changes in genetic variation are associated with passive dispersal. Considerable genetic divergence was evident among Heterocope populations, for example, entirely due to differences in shared gene frequencies and there was a significant loss of heterozygosity in populations derived from the ancestors in glacial refugia. Similar genetic studies over broad geographic areas in other terrestrial taxa were unable to detect such significant losses (Schwaegerle and Schaal,

1979; Berlocher, 1984; Parkin and Cole, 1985; Baker and Moeed, 1987; Eastcal, 1985) and the copepods may prove ideal for the evaluation of population genetics theory. For example, Wright's (1943) island model, which assumes discrete populations and symetric gene flow, is difficult to duplicate in natural populations. The theory, however, has been widely applied to genetic data to quantify dispersal. The present study, using aquatic taxa in discrete demes, has demonstrated the danger of uncritical applications of this theory, without concurrent knowledge of size and age of populations.

In summary, further genetic studies on copepods are likely to provide a basis for the revision of the systematics of the group at both the species and higher taxonomic levels. Such revisions will lead to an important extension in our understanding of distributional patterns of extant taxa. Genetic data will also permit inferences about the likely routes of dispersal taken by postglacial colonists. From an evolutionary standpoint copepods are likely also to serve as a model group in examinations of the evolutionary impact of population differentiation. Their discrete population structure more closely resembles patterns frequently modeled by theoreticians, unlike many other organisms which have been examined in empirical studies.

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APPENDIX I

Descriptions of collecting sites and copepod species obtained for electrophoretic analysis.

A. Eastern arctic and subarctic localities

(1) Churchill, Manitoba 58° 47'N 94° 11'W

TW2: At terminus of Twin Lakes Road, across from the fire pit.
Heterocope septentrionalis

Causeway 1/Causeway 2: On east (1) and west (2) side of Twin Lakes Road, at point where road exits the forest and enters wide tundra plain (the causeway).
Hesperodiaptomus wilsonae

All other ponds in tundra plain and on rock bluffs along Hudson Bay coastline as described in Good (1981)
Heterocope septentrionalis
Hesperodiaptomus victoriaensis
Hesperodiaptomus nearcticus
Leptodiaptomus tyrrelli
Eurytemora composita

(2) Eskimo Point (Arviat), District of Keewatin N.W.T. 61°7'N 94°3'W

12 ponds located in immediate vicinity of townsite.
Heterocope septentrionalis
Hesperodiaptomus nearcticus
Hesperodiaptomus victoriaensis
Arctodiaptomus bacillifer

(3) Rankin Inlet, District of Keewatin N.W.T. 62°49'N 92°5'W

12 ponds in immediate vicinity of townsite and one pond (RT8) 4km northwest of town on road to Char River, at bend in road before river ford.
Heterocope septentrionalis (RT8 only)
Hesperodiaptomus nearcticus
Hesperodiaptomus victoriaensis
Arctodiaptomus bacillifer

(4) Igloolik, District of Franklin N.W.T. (collected by Paul Hebert)

9 ponds on island described by Welder et al. (1987)
Hesperodiaptomus eigeni
Eurytemora composita
Cyclops canadensis

(5) Cape Fe. (collected by Paul Hebert)

Pond #s 2, 4 and 5- tundra ponds
Hesperodiaptomus eigani

B. Western arctic localities

(6) Inuvik, District of Mackenzie N.W.T. 68°21'N 133°43'W

5 ponds in immediate vicinity of townsite and one pond 20 km east on Dempster Highway
Heterocope septentrionalis
Leptodiaptomus pribilofensis

(7) Tuktoyaktuk, District of Mackenzie N.W.T. 69°27'N 133°2'W

6 ponds in immediate vicinity of townsite and road leading to pingos, 1 pond near largest pingo.
Heterocope septentrionalis
Hesperodiaptomus arcticus
Limnocalanus johanseni
Arctodiaptomus bacillifer

4 ponds on peninsula
Heterocope septentrionalis
Hesperodiaptomus arcticus

(8) Liverpool Bay, District of Mackenzie N.W.T. 69°49'N 129°25'W

3 deep tundra ponds.
Heterocope septentrionalis

(9) Nicholson Point, District of Mackenzie N.W.T. 69°57'N 128°59'W

2 ponds next to road between Dew line installation and airstrip, at the fuel dump.
Hesperodiaptomus nearcticus

(10) Paulatuk, District of Mackenzie N.W.T. 69°22'N 124°2'W

3 ponds on peninsula between townsite and channel markers, 1 pond next to airstrip.
Heterocope septentrionalis
Hesperodiaptomus nearcticus

(11) Herschel Island, Provincial Park 69°16'N 138°55'W

2 ponds, 1 on north point of island, 1 pond on slope adjacent to Thetis Bay
Heterocope septentrionalis

(12) Firth River, Yukon 69°20'N 139°15'W

2 ponds, in deltaic fan near coastline
Heterocope septentrionalis

(13) Mackenzie Delta, District of Mackenzie N.W.T.

2 ponds on raised platform on western margin of the delta, near Tununuk Point
Heterocope septentrionalis
Hesperodiaptomus arcticus

C. Rocky Mountain localities

(14) Kananaskis Country, Alberta

Teardrop Pond (Anderson, 1968) 50°12'N 114°27'W Large circular pond at summit of Johnson Creek Pass (el. 2030m asl), shallow, weedy.
Leptodiaptomus tyrrelli-Sept 2/88.

(15) Banff National Park, Alberta

Ptarmigan Lake--(B 195; Anderson, 1974) Large, deep, clear lake at summit of Boulder Pass (2324m asl); rocky bottom.
Leptodiaptomus tyrrelli-Sept 4/88.

Baker Lake--(B 198; Anderson, 1978) Large, deep, turbid lake just below Ptarmigan Lake (2207m asl); silt and clay bottom.
Hesperodiaptomus nearcticus
Leptodiaptomus tyrrelli-Sept 4/88.

Brachiopod Pond--(B 200?; Anderson, 1978) Small, deep pond fed by underground spring (2270m asl); unconsolidated detritus bottom; narrow vegetation mat border.
Hesperodiaptomus nearcticus-Sept 4/88.

Bow Pass Pond--Small tundra like pond at summit of Bow Pass, on east side of Icefields Hwy.; very deep with unconsolidated detritus bottom and narrow mat border.
Aglaodiaptomus leptopus-Sept 10/88.

(16) Jasper National Park

Cavell Lake--(J 778; Anderson, 1978) Large, shallow pond fed by Angel Glacier (2017m asl); turbid; boulder bottom.
Hesperodiaptomus nearcticus-Sept 9/88.

Leach Lake--(J 295?; Anderson, 1978) Large, shallow, clear pond (1220m asl); flagstone bottom with unconsolidated detritus layer.
Aglaodiaptomus leptopus
Leptodiaptomus tyrrelli-Sept 10/88.

(17) Northern BC (collected by Paul Hebert)

BC 12--Small pond in aspen grove 40 km northeast of Hudson's Hope; probably man made pond.
Aglaodiaptomus leptopus-June 17/88

BC 16--50 km north of Prince George, 1 m deep beaver pond
Aglaodiaptomus leptopus-June 17/88

(18) Cypress Hills Provincial Park (collected by Paul Hebert)

Alb 9--Large, shallow pond, 2 km from northern boundary of park
Hesperodiaptomus nevadensis June 14/88

D. Prairie localities

(19) Saskatoon, Saskatchewan (collected by Guy Melville)

4 Mel--Large semipermanent pond 2km east of Saskatoon, choked with macrophytes and filamentous algae.
Hesperodiaptomus churchillensis-May 29/88.

5 Mel--Small pond, 100m south of and probably connected in some years to 4 Mel.
Hesperodiaptomus churchillensis-May 29/88.

E Mel--Small, temporary pond, 2km east of Saskatoon, 2-3 km south of 4 Mel and 5 Mel.
Hesperodiaptomus churchillensis-May 29/88.

F Mel--Temporary pond, formerlly connected to E Mel; choked with macrophytes at midsummer.
Hesperodiaptomus churchillensis-May 29/88.

(20) Wisconsin (collected by John Havel)

Big Frank Lake--Bog lake near Boulder Junction, northern Wisconsin
Aglaodiaptomus leptopus

E. Kent and Essex Counties, Ontario

(21) Rondeau Park--Pond in depression between ancient beach ridges, north of Interpretive Centre Rd. and south of old group camping area.
Acanthocyclops americanus
A. robustus

(22) Ashers--Ditch on north side of Raleigh Rd. XVI, west of Four Rod Rd. near Asher's residence.
Acanthocyclops parcus

(23) Kendall--Pond in woodlot on southwest corner of Kendall Rd. and Raleigh Rd. XVI.
Acanthocyclops parcus

(24) McGeachy--Large embayment of Lake Erie, now a shallow marshy pond, at junction of Erieau and Erie Beach Rds.
Acanthocyclops americanus
A. robustus

(25) Baseline--Shallow pond on west side of Sandwich South Rd. XII, south of Baseline Rd. halfway to 401 highway.
Acanthocyclops parcus

(26) Devon Woods--Small pond near parking lot to Devon Woods in south Windsor.
Acanthocyclops parcus
Diacyclops navus

(27) Lake St. Clair--Sampled near mouth of Thames River at lighthouse.
Acanthocyclops brevispinosus

F. Britain (U.K.)

(28) Ravenstone--Shallow farm pond at edge of village of Ravenstone, between Loughborough and Ashby.
Acanthocyclops vernalis

(29) Windmill--
Acanthocyclops vernalis

(30) Oughtershaw--Tarn on plateau above Oughtershaw, Ordnance Survey Sheet 98, SD882821
Acanthocyclops vernalis

(31) Shardlow--Large deep pond, on road leading to electric generating station, south of A6 carriageway near intersection with M1 motorway.
Acanthocyclops robustus

APPENDIX III

A. Autosomal locus independence matrix for *Heterocope septentrionalis*. Matrix values are Williams adjusted G (Sokal and Rohlf, 1981) and degrees of freedom (in parentheses). +=other tests for independence conducted but not reported, highest G-value in table. X=no test done.

	PGI	PGM	AMY	MPI	AO	XDH	MDH
PGI	---						
PGM	1.05 (4)	---					
AMY	X	2.55 (4)	---				
MPI	9.42 (4)	7.57++ (4)	1.73 (4)	---			
AO	X	1.34+ (4)	7.59 (4)	5.32+ (4)	---		
XDH	X	1.80 (4)	X	0.71 (4)	6.54 (4)	---	
MDH	7.44 (4)	2.94++ (4)	0.90 (4)	7.58++ (4)	1.10+ (4)	6.92 (4)	---

B. Autosomal locus independence matrix for *Hesperodiaptomus* spp. Matrix values are Williams adjusted G (Sokal and Rohlf, 1981) and degrees of freedom (in parentheses). a=*H. arcticus*; c=*H. churchillensis*. X=no test done.

	MDH	PGM	MPI	GOT	XDH	PGI	APK-4
MDH	---						
PGM	0.76a (2)	---					
MPI	2.82a (1)	4.15c 2.51a (4c,2a)	---				
GOT	2.61a (2)	6.29a (4)	0.20a (2)	---			
XDH	2.78a (2)	5.02a (4)	2.43a (2)	2.10a (4)	---		
PGI	X	5.85c (4)	5.23c (4)	X	X	---	
APK-4	X	4.90c 2.00c (4)	X	X	X	4.58c (4)	---

Appendix IV

BASIC program to simulate the decay of gene frequency differences resulting from founding populations. Program was compiled for execution into machine language using Bascom by IBM.

```
100 DIM Q(101),F(25,105):CLS
110 INPUT"  WHAT IS THE INITIAL FREQUENCY OF THE SOURCE POPULATION";PI
120 INPUT"  How many founders(=dispersers in subsequent generations)  ";M
130 INPUT"  What is the size of populations desired";N
135 INPUT"  How many populations do you want founded (<100)";SUB
140 INPUT"  How many replicates of the simulation do you want";REPL
150 INPUT"  What is the name of the output file--Don't forget the drive
            and extension.
            Be sure your disk is loaded before you begin";OTF$
155 TMER=VAL(MID$(TIME$,7,2))
160 RANDOMIZE TMER
165 VAR=0:FSUM=0
170 FOR R=1 TO REPL
175 Q2SUM=0:QSUM=0:P2SUM=0
180 FOR S=1 TO SUB
190 QAT=0
220 GOSUB 1000
240 Q(S)=QAT/(M*2):QSUM=QSUM+Q(S)
250 NEXT S
300 QMN=QSUM/SUB:PMN=1-QMN
320 FOR X=1 TO SUB:P=1-Q(X)
330 Q2SUM=Q2SUM+Q(X)^2:P2SUM=P2SUM+P^2
340 NEXT X
350 Q2M=Q2SUM/SUB:P2M=P2SUM/SUB
360 HS=(1-(Q2M+P2M)):HT=(1-(QMN^2+PMN^2))
370 IF HT=0 THEN F=0:GOTO 390
380 F=(HT-HS)/HT
390 F(0,R)=F
410 FOR GEN=1 TO 2000
420 G2=G2 + 1
430 FOR S=1 TO SUB
440 QAT=0
450 GOSUB 2000
460 Q1=(QAT+(Q(S)*N*2))/((N+M)*2)
470 Q(S)=Q1
480 NEXT S
500 GOSUB 3000
510 NEXT GEN
520 NEXT R
590 GOSUB 4000
600 PRINT"The simulation has ended":END
```

```
1000 FOR I=1 TO M
1010 MC=RND
1020 IF MC<=PI^2+(2*PI*(1-PI)) THEN QAT=QAT + 1
1030 IF MC<=PI^2 THEN QAT=QAT + 1
1040 NEXT I
1050 RETURN
2000 FOR I=1 TO M
2010 MC=RND
2020 IF MC<=QMN^2 + 2*QMN*(1-QMN)-(F*QMN*(1-QMN)) THEN QAT=QAT + 1
2030 IF MC<=QMN^2 + F*QMN*(1-QMN) THEN QAT=QAT + 1
2040 NEXT I
2050 RETURN
3000 QSUM=0:Q2SUM=0:P2SUM=0
3010 FOR X=1 TO SUB:P=1-Q(X)
3020 Q2SUM=Q2SUM+Q(X)^2:P2SUM=P2SUM+P^2
3030 QSUM=QSUM + Q(X)
3040 NEXT X
3045 QMN=QSUM/SUB:PMN=1-QMN
3050 Q2M=Q2SUM/SUB:P2M=P2SUM/SUB
3060 HS=(1-(Q2M+P2M)):HT=(1-(QMN^2+PMN^2))
3070 IF HT=0 THEN F=0:GOTO 3090
3080 F=(HT-HS)/HT
3090 IF G2=100 THEN GOTO 3500
3100 RETURN
3500 G=GEN/100:F(G,R)=F:G2=0
3510 RETURN
4000 OPEN OTF$ FOR OUTPUT AS #1
4010 FOR G=0 TO 20
4020 PRINT#1,"Generation=";G*100:FSUM=0:VAR=0
4030 FOR R=1 TO REPL
4040 PRINT#1 F(G,R)
4050 FSUM=FSUM + F(G,R)
4060 VAR=VAR+(F(G,R)-FM)^2
4070 NEXT R
4080 FM=FSUM/REPL
4090 FVAR=VAR/REPL
4100 PRINT#1, USING" mean Fst=#.### var.=#.####";FM, FVAR
4200 NEXT G
```

Appendix V

Matrices of genetic identity (Nei, 1978) for Heterocope septentrionalis (A) and Hesperodiaptomus arcticus s. l. (B) from BIOSYS-1.

A.

POPULATION	1	2	3	4	5
1 FIRTH RIVER YK	*****				
2 HERSCHEL IS. YK.	0.937	*****			
3 MACKENZIE DELTA	0.891	0.894	*****		
4 MACKENZIE DELTA	0.871	0.895	0.992	*****	
5 TUKTOYAKTUK NWT	0.769	0.842	0.884	0.922	*****
6 TUKTOYAKTUK NWT	0.852	0.845	0.910	0.917	0.936
7 TUKTOYAKTUK NWT	0.844	0.854	0.916	0.940	0.961
8 TUKTOYAKTUK NWT	0.862	0.870	0.907	0.908	0.904
9 INUVIK NWT	0.910	0.867	0.904	0.889	0.829
10 INUVIK NWT	0.914	0.854	0.914	0.894	0.815
11 INUVIK NWT	0.921	0.850	0.939	0.915	0.813
12 LIVERPOOL BAY	0.831	0.816	0.891	0.890	0.881
13 PAULATUK	0.833	0.808	0.859	0.870	0.855
14 PAULATUK	0.814	0.774	0.871	0.880	0.855
15 PAULATUK	0.793	0.790	0.855	0.869	0.876
16 CHURCHILL MAN.	0.836	0.806	0.833	0.859	0.845
17 CHURCHILL MAN.	0.818	0.791	0.849	0.877	0.875
18 CHURCHILL MAN.	0.817	0.792	0.870	0.896	0.901
19 CHURCHILL MAN.	0.820	0.775	0.860	0.888	0.898
20 ESKIMO/RANKIN	0.791	0.724	0.795	0.812	0.780
21 ESKIMO POINT	0.793	0.729	0.820	0.838	0.805
22 ESKIMO POINT	0.801	0.733	0.804	0.822	0.788

POPULATION	6	7	8	9	10	11
1 FIRTH RIVER YK						
2 HERSCHEL IS. YK.						
3 MACKENZIE DELTA						
4 MACKENZIE DELTA						
5 TUKTOYAKTUK NWT						
6 TUKTOYAKTUK NWT	*****					
7 TUKTOYAKTUK NWT	0.963	*****				
8 TUKTOYAKTUK NWT	0.934	0.930	*****			
9 INUVIK NWT	0.881	0.890	0.949	*****		
10 INUVIK NWT	0.895	0.873	0.952	0.985	*****	
11 INUVIK NWT	0.892	0.881	0.928	0.971	0.990	*****
12 LIVERPOOL BAY	0.916	0.936	0.888	0.901	0.896	0.906
13 PAULATUK	0.887	0.913	0.870	0.929	0.904	0.907
14 PAULATUK	0.895	0.917	0.889	0.907	0.900	0.915
15 PAULATUK	0.908	0.909	0.920	0.914	0.910	0.895
16 CHURCHILL MAN.	0.846	0.912	0.852	0.876	0.861	0.864
17 CHURCHILL MAN.	0.883	0.933	0.896	0.904	0.895	0.884
18 CHURCHILL MAN.	0.925	0.945	0.939	0.902	0.910	0.895
19 CHURCHILL MAN.	0.896	0.944	0.904	0.895	0.894	0.892
20 ESKIMO/RANKIN	0.831	0.855	0.808	0.855	0.843	0.840
21 ESKIMO POINT	0.863	0.870	0.835	0.849	0.849	0.848
22 ESKIMO POINT	0.851	0.865	0.823	0.852	0.845	0.840

	POPULATION	12	13	14	15
1	FIRTH RIVER YK				
2	HERSCHEL IS. YK.				
3	MACKENZIE DELTA				
4	MACKENZIE DELTA				
5	TUKTOYAKTUK NWT				
6	TUKTOYAKTUK NWT				
7	TUKTOYAKTUK NWT				
8	TUKTOYAKTUK NWT				
9	INUVIK NWT				
10	INUVIK NWT				
11	INUVIK NWT				
12	LIVERPOOL BAY	*****			
13	PAULATUK	0.965	*****		
14	PAULATUK	0.961	0.976	*****	
15	PAULATUK	0.952	0.964	0.975	*****
16	CHURCHILL MAN.	0.906	0.921	0.910	0.889
17	CHURCHILL MAN.	0.919	0.935	0.926	0.926
18	CHURCHILL MAN.	0.931	0.924	0.951	0.962
19	CHURCHILL MAN.	0.915	0.920	0.936	0.921
20	ESKIMO/RANKIN	0.849	0.898	0.874	0.850
21	ESKIMO POINT	0.854	0.887	0.891	0.868
22	ESKIMO POINT	0.852	0.887	0.873	0.849

POPULATION	16	17	18	19	20	21
16 CHURCHILL MAN.	*****					
17 CHURCHILL MAN.	0.957	*****				
18 CHURCHILL MAN.	0.943	0.973	*****			
19 CHURCHILL MAN.	0.970	0.986	0.983	*****		
20 ESKIMO/RANKIN	0.921	0.924	0.890	0.903	*****	
21 ESKIMO POINT	0.904	0.914	0.914	0.916	0.988	*****
22 ESKIMO POINT	0.907	0.914	0.899	0.907	0.994	0.995

B.

POPULATION	1	2	3	4	5
1 TUK PENINSULA	*****				
2 NICHOLSON	0.973	*****			
3 NICHOLSON 2	0.926	0.960	*****		
4 BANFF BAKER	0.720	0.769	0.821	*****	
5 BANFF BRACHIOPOD	0.794	0.809	0.843	0.931	*****
6 JASPER CAVELL	0.816	0.846	0.836	0.913	0.923
7 PAULATUK 2	0.864	0.897	0.940	0.837	0.870
8 PAULATUK 3	0.817	0.856	0.895	0.886	0.917
9 CHURCHILL T92	0.860	0.886	0.858	0.854	0.918
10 CHURCHILL C44	0.855	0.908	0.896	0.850	0.878
11 CHURCHILL A21	0.876	0.920	0.884	0.858	0.891
12 CHURCHILL C90	0.692	0.732	0.739	0.696	0.745
13 CHURCHILL C107	0.710	0.730	0.695	0.707	0.787
14 ESKIMO POINT 12	0.805	0.850	0.853	0.802	0.845
15 ESKIMO POINT 13	0.798	0.845	0.861	0.817	0.857
16 RANKIN INLET 5	0.822	0.838	0.813	0.802	0.864
17 RANKIN INLET 8	0.808	0.809	0.803	0.818	0.913
18 DELTA 2	0.499	0.514	0.508	0.450	0.468
19 TUKTOYAKTUK 6	0.539	0.549	0.532	0.489	0.517
20 TUKTOYAKTUK 11	0.520	0.533	0.522	0.470	0.493
21 TUKTOYAKTUK 13	0.518	0.531	0.521	0.468	0.489
22 TUKTOYAKTUK 14	0.534	0.545	0.529	0.484	0.510
23 TUK EAST HARBOUR	0.542	0.554	0.538	0.493	0.517
24 SASKATOON FMEL	0.728	0.735	0.742	0.627	0.689
25 SASKATOON 5MEL	0.744	0.757	0.771	0.660	0.719
26 SASKATOON GMEL	0.723	0.735	0.743	0.630	0.690
27 SASKATOON 4MEL	0.741	0.758	0.778	0.668	0.726

	POPULATION	6	7	8	9	10	11
1	TUK PENINSULA						
2	NICHOLSON						
3	NICHOLSON 2						
4	BANFF BAKER						
5	BANFF BRACHIOPOD						
6	JASPER CAVELL	*****					
7	PAULATUK 2	0.879	*****				
8	PAULATUK 3	0.928	0.969	*****			
9	CHURCHILL T92	0.941	0.916	0.963	*****		
10	CHURCHILL C44	0.899	0.883	0.932	0.948	*****	
11	CHURCHILL A21	0.927	0.884	0.932	0.972	0.991	*****
12	CHURCHILL C90	0.741	0.755	0.804	0.808	0.839	0.825
13	CHURCHILL C107	0.798	0.722	0.770	0.809	0.782	0.800
14	ESKIMO POINT 12	0.849	0.899	0.950	0.958	0.959	0.950
15	ESKIMO POINT 13	0.858	0.909	0.960	0.956	0.959	0.946
16	RANKIN INLET 5	0.894	0.900	0.947	0.989	0.906	0.932
17	RANKIN INLET 6	0.915	0.904	0.948	0.976	0.869	0.902
18	DELTA 2	0.504	0.512	0.502	0.508	0.494	0.521
19	TUKTOYAKTUK 6	0.558	0.548	0.537	0.554	0.515	0.553
20	TUKTOYAKTUK 11	0.532	0.531	0.521	0.532	0.506	0.538
21	TUKTOYAKTUK 13	0.528	0.529	0.519	0.529	0.505	0.536
22	TUKTOYAKTUK 14	0.552	0.543	0.533	0.548	0.512	0.548
23	TUK EAST HARBOUR	0.558	0.554	0.543	0.556	0.526	0.560
24	SASKATOON FMEL	0.692	0.790	0.800	0.809	0.785	0.785
25	SASKATOON 5MEL	0.716	0.817	0.830	0.831	0.814	0.809
26	SASKATOON GMEL	0.696	0.790	0.802	0.809	0.790	0.786
27	SASKATOON 4MEL	0.725	0.830	0.841	0.838	0.819	0.812

POPULATION	18	19	20	21	22	23
1 TUK PENINSULA						
2 NICHOLSON						
3 NICHOLSON 2						
4 BANFF BAKER						
5 BANFF BRACHIOPOD						
6 JASPER CAVELL						
7 PAULATUK 2						
8 PAULATUK 3						
9 CHURCHILL T92						
10 CHURCHILL C44						
11 CHURCHILL A21						
12 CHURCHILL C90						
13 CHURCHILL C107						
14 ESKIMO POINT 12						
15 ESKIMO POINT 13						
16 RANKIN INLET 5						
17 RANKIN INLET 8						
18 DELTA 2	*****					
19 TUKTOYAKTUK 6	0.967	*****				
20 TUKTOYAKTUK 11	0.983	0.997	*****			
21 TUKTOYAKTUK 13	0.987	0.980	0.987	*****		
22 TUKTOYAKTUK 14	0.972	1.000	0.998	0.982	*****	
23 TUK EAST HARBOUR	0.989	0.986	0.988	0.990	0.986	*****
24 SASKATOON FMEL	0.409	0.438	0.428	0.419	0.434	0.441
25 SASKATOON 5MEL	0.424	0.450	0.442	0.434	0.447	0.453
26 SASKATOON GMEL	0.410	0.442	0.432	0.420	0.439	0.442
27 SASKATOON 4MEL	0.412	0.433	0.425	0.421	0.431	0.439

	POPULATION	24	25	26
1	TUK PENINSULA			
2	NICHOLSON			
3	NICHOLSON 2			
4	BANFF BAKER			
5	BANFF BRACHIOPOD			
6	JASPER CAVELL			
7	PAULATUK 2			
8	PAULATUK 3			
9	CHURCHILL T92			
10	CHURCHILL C44			
11	CHURCHILL A21			
12	CHURCHILL C90			
13	CHURCHILL C107			
14	ESKIMO POINT 12			
15	ESKIMO POINT 13			
16	RANKIN INLET 5			
17	RANKIN INLET 8			
18	DELTA 2			
19	TUKTOYAKTUK 6			
20	TUKTOYAKTUK 11			
21	TUKTOYAKTUK 13			
22	TUKTOYAKTUK 14			
23	TUK EAST HARBOUR			
24	SASKATOON FMEL	*****		
25	SASKATOON 5MEL	0.996	*****	
26	SASKATOON GMEL	0.999	0.998	*****
27	SASKATCON 4MEL	0.991	1.000	0.992

APPENDIX VI

Field survey, electrophoretic and ephemerality results for Bluff C ponds examined for distribution of *Hesperodiaptomus* isolates. (N=Isolate N, C=Isolate C, Proportions and Ephemerality described in Chapter V Methods)

Pond #	Isolates Observed in Field Survey (Electrophoretic Proportion)	Ephemerality 1984	Ephemerality 1985
1	N	13	14
2	C	5	14
3	C	6	14
8a	C	NA	12
9a	C	6	14
13	N (.80) C (.20)	10	14
15	C	6	12
16	N (.10) C (.90)	5	12
20	N	10	14
23	N	13	14
27	C	10	12
28	N	13	14
29	C	6	12
36	N (1.0)	10	14
37	N	6	14
39	N	10	14
41	N (.05) C (.95)	6	12
42	C	10	12
43	C	5	10
44	N (1.0)	13	14
45	N (1.0)	13	14
45a	N	13	14
47	N	13	14
50	N	10	14
51	N (.65) C (.35)	6	12
52	N	10	14
52a	N	13	14
53	N	13	14
54	C	5	12
57	C (1.0)	5	10
59	N C	10	12
60	C	6	12
61	N (.15) C (.85)	6	12
63	C	10	10
64	C	6	14
66	N	6	14
70	C	5	10
82	N	13	14
83	N	13	14
84	N (1.0)	13	14
88	C	5	10
89	C (1.0)	6	12
90	C (1.0)	6	12
98	C (1.0)	10	10
100	N (1.0)	13	14

Appendix VI cont'd

Pond #	Isolates Observed in Field Survey (Electrophoretic Proportion)	Ephemerality 1984	1985
107	C	5	10
112	N	13	14
132	N (1.0)	6	10
149	N (1.0)	13	14
156	N (.14) C (.86)	13	14

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EDUCATION

1971 Chatham Kent Secondary School
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GRANTS/AWARDS

NSERC Postdoctoral Fellowship (1989-Cornell University)
Department of Indian Affairs and Northern Development,
Northern Training Grants (1984-1989)
Northern Conference Travel Award (1986)
Department of Energy Mines and Resources,
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Ontario Graduate Scholarship (1985-86)
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Chatham Metal Finishing Limited: Aug. 1971-July 1981.
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Lake Erie Fisheries Research Unit: May-Sept. 1983.
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SCIENTIFIC COMMUNICATIONS

1989 Section of Ecology and Systematics, Cornell University, NY
"Non-equilibrium gene frequency divergence: implications for the estimation of gene flow."

1988 Department of Biology, University of Houston, Texas
"Genetic differentiation and gene flow among populations of arctic calanoid copepods."

1987 Third International Conference on Copepoda, Imperial College, London, England
"Genetic differentiation of copepods at a low-arctic site."
"Biochemical systematics in Copepoda."

1987 Ontario Ecology and Ethology Colloquium, University of Ottawa
"Genetic differentiation of freshwater pond copepods at a low arctic site."

1986 Student Conference for Northern Studies, Association of Canadian Universities for Northern Studies, Ottawa
"Biochemical differences between two related species of Leptodiaptomus, (Copepoda)."

1986 Midwest Ecology and Evolution Conference, University of Michigan, Ann Arbor
"Genetic differentiation among copepod populations at a low arctic site."

1984 Ontario Archaeological Society, Windsor Chapter
"Investigations of the prehistoric Thule culture at Peale Point (KkDo-1) N.W.T."

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